PHYTOREMEDIATION OF ARSENIC AND THE IMMOBILIZATION OF LEAD IN SOIL: THE IMPACTS OF APATITE PARTICLE SIZE

A Thesis

by

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ABSTRACT

Apatite was added to a soil highly contaminated with As and Pb to enhance As uptake by the hyperaccumulating fern, *Pteris cretica*. Arsenic and lead co-contamination is common in smelter soils and can be a major source of environmental harm. *In situ* chemical stabilization of contaminants can be an acceptable remediation option, but simultaneous immobilization of Pb and As faces serious obstacles. The two contaminants have antagonistic immobilization chemistries: the effective use of P to immobilize Pb greatly mobilizes As. However, this effect can be exploited if the mobilized As is assimilated by As hyperaccumulating plants, such as members of the *Pteris* species. Apatite is an effective source of phosphorus when used with *Pteris* arsenic hyperaccumulators but the effect of particle size has not been studied. Changes in soil and plant chemistry were studied for two concentrations of total soil As (750 mg/kg and 1,500 mg As /kg soil); three particle sizes of added apatite (500 μm-250 μm, 250 μm-105 μm, <105 μm); and three molar ratios of P:As (1:1, 1:2, 1:5). The treatments were found to have a significant impact on both the arsenic concentrations in the plants and plant biomass. In addition, IVBA analysis of the soil showed a reduction in the amount of arsenic found to be bioaccessible. Plant phosphorus concentrations and plant arsenic concentrations were correlated with indications that phosphorus is promoting arsenic uptake and plant growth.

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1. INTRODUCTION

1.1. General Background

The U.S. Environmental Protection Agency (EPA) National Priority List (NPL) documents 1,335 contaminated sites in the United States as of November 08, 2019, that are eligible for long term remedial action as detailed under the federal Superfund program [55]. These sites contain hazardous substances that pose an existing or imminent threat to humans, animals, and the environment. From the myriad of hazardous substances found at Superfund sites, arsenic is categorized as the substance of highest priority according the Agency of Toxic Substances and Disease Registry (ATSDR) [56]. Being a group A carcinogen, elevated exposure to arsenic can lead to cancer and other health risks.

The Jacob Smelter Superfund site is located near Stockton, Utah, and covers about eight square miles. The source of the contamination is from smelting and mining of gold, silver, copper, lead, and zinc that occurred primarily from the 1860s through the 1870s. A total of nine different smelters existed in this area during this time, all contributing to the contamination. Throughout this period, waste was generated in the form of heavy-metal contaminated soils, mill tailings, and smelter wastes.

In 1998, the EPA conducted a removal assessment which showed that the area had high levels of arsenic and lead they posed a significant risk to human health. In 2000, the site was officially added to the national priorities list as a Superfund site requiring immediate remediation efforts. The primary pathway for exposure to the toxins is through inhalation and ingestion of contaminated soil and dust particles. Developing

children are the segment of the population most vulnerable to the health impacts and are susceptible to arsenic and lead poisoning. Contaminated areas extend into residential areas as well as non-residential [57]. The Utah Department of Environmental Quality (UDEQ), working with the EPA, forthfilled a record of decision (ROD) that emphasized that "lead and arsenic are identified in concentrations that pose a significant risk to human health and environment" [58].

The greatest concentrations of contamination at the site exceed 150,000 mg kg^{-1} of Pb. The contamination is mostly limited to the upper 45 cm of the soil. Based on US EPA standard protocols for assessing risk of cancer and non- cancerous diseases, an action level of 500 mg kg^{-1} Pb and 100 mg kg^{-1} As was used for residential surface soil; 800 mg kg^{-1} Pb for residential subsurface soil; 3000 mg kg^{-1} in both surface and subsurface soil for recreational areas; and 2,200 mg kg^{-1} of Pb for surface and subsurface soils in commercial and industrial areas [57]. To achieve these levels, the record of decision by the EPA is to excavate the contaminated soil to a maximum of 45 cm and place the soil in an offsite land-fill. The projected time is 12 months at a cost of $9,647,000.

Conventional active remediation methods for metal contaminated soils include acid washing, acid extraction, flushing, vitrification, and immobilization. An alternative method that can be employed is phytoremediation: removal of the contaminants, in this case arsenic, from the soil with minimal disturbance of the soil. Phytoremediation of metals is primarily based on the use of hyperaccumulators to assimilate a specific contaminant. Hyperaccumulators have been found for several metals including

selenium, nickel, copper, chromium, titanium, cadmium, zinc, manganese and arsenic [81].

Phosphorus as an amendment has the added benefit of precipitating lead from the soil solution by forming insoluble fluoropyromorphite-like minerals [59]. In soils with very high concentrations of Pb, the extreme concentrations of P needed to amend such soils have the potential to cause salt toxicity in plants. At the same time, the large quantities of P will cause arsenic to desorb from soil exchange sites and become bioaccessible to arsenic hyperaccumulators. Although sparingly soluble in alkaline soils, apatite can be solubilized by the roots of *Pteris* species, and the released P might be able to impact the high levels of arsenic and lead in contaminated soils. Particle size would play a large role in the speed of dissolution of the apatite and affect its viability as a remediation tool.

1.1.1. Specific Objectives

1. Determine the effect of apatite particle size and P:As molar ratio on arsenic uptake by *Pteris cretica* as well as As and Pb the bioaccessibility of determined by the IVBA test.
2. Evaluate the ability of *Pteris cretica* to solubilize apatite in the soil, thus increasing the bioavailability of arsenic.

1.1.2. Hypotheses:

1. Lead bioaccessibility will decrease with decreasing apatite particle size, and arsenic bioaccessibility will increase with decreasing apatite particle size,
2. Additions of apatite will significantly increase the plant uptake of arsenic.
3. Arsenic uptake by *Pteris cretica* will be correlated to $NaHCO_3$ extractable arsenic.

2. LITERATURE REVIEW

2.1. *Pteris* Hyperaccumulators

2.1.1. *Pteris vittata*

Several *Pteris* species are known to hyperaccumulate arsenic from soils. By far the most well know is *Pteris vittata* which was discovered to be an As hyperaccumulator in 2001. This species assimilates large amounts of arsenic, as high as 2% dry weight mass, without showing the usual symptoms of arsenic toxicity [1].

Arsenic is non-essential and generally toxic to most plants. Roots are the first plant tissue to be exposed to soil arsenic, where the metalloid inhibits root extension and proliferation. Upon translocation to the shoot, arsenic can severely limit growth of susceptible plants by slowing or even stopping expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production. At sufficiently high concentrations, arsenic interferes with critical metabolic processes, which can lead to death [54]. Most plants possess mechanisms that limit the spread of arsenic to their roots. However, *P. vittata* is capable of handling high levels of arsenic because of its unusual mechanisms to reduce the toxicity of this metalloid once absorbed. Instead of excluding arsenic when assimilating nutrients, which is the most common way plants avoid arsenic toxicity, *P. vittata* accepts arsenic in both arsenate and arsenite forms and then transports it to the fronds in the reduced As(III) state [2].

Reduction of As(V) to As(III) is vital in the plant's ability to mitigate the toxicity of arsenic. Because arsenate is a chemical analog of phosphate, it can replace phosphate

in ATP synthesis and interfere with phosphate metabolism. Once reduced, As(III) becomes complexed with organic ligands within plant root cells, translocated to the fronds, and sequestered in the vacuoles [10]. The plant also keeps a high phosphorus-to-arsenic ratio in the roots to maintain normal metabolic processes with minimal disruptions from As. The high P:As ratio is vital in the roots because the majority of the arsenic found in the roots is arsenate, the form most toxic to plants. The reduction of arsenate to arsenite takes place in the roots, and inorganic arsenite is strongly favored (93-98%) for transport in the xylem sap [19].

Transport of metals and metalloids to the plants' above ground biomass is a common phenomenon of hyperaccumulating species; plants that are simply tolerant to toxic metal(loids) store the majority of the absorbed metal(loids) in their roots. A high transfer factor, the concentration ratio of a particular element in the plant's above ground biomass as compared to its roots, is characteristic of hyperaccumulating plants [3]. The bioaccumulation factor, the ability of the plant to concentrate the element from the soil, is also an important characteristic of plants used for phytoremediation. Phytoremediation is only possible when the plant can transport the toxic metal or metalloid out of the soil and into the above ground biomass that can then be harvested and disposed [4].

Due to the robust growth of the hyperaccumulating plant as well as its strong affinity for absorbing arsenic, significant quantities of arsenic can be removed from the soil in a relatively short time. The mass of arsenic removed by the plant is influenced by soil texture, environment, soil chemical properties, and form of arsenic in the soil. Some trials have shown decreases in soil concentrations as high as 25 mg kg^{-1} [5]. Successive

harvest and growth cycles can significantly increase the levels of contaminants being removed from the soil. This is the common strategy for phytoremediation and the most effective way to return soil levels to acceptable ranges.

Native to alkaline and calcareous soils, *P. vittata* has developed numerous strategies to obtain nutrients in an environment that is often low in bioavailable nutrients. The availability of some nutrients is increased by secreting organic acids that dissolve nutrients in the solid phase or sorbed to soil particles, bringing them into solution and making them available for uptake [5]. Although not a plant nutrient, As can be mobilized by many of these same mechanisms. Soils low in available phosphorus encourage the plant to exude even more organic acids. Uniquely, the hyperaccumulating plants of this genus also exude more organic acids in the presence of arsenic [6].

2.1.2. *Pteris cretica*

The discovery of *Pteris vittata* led to a search for other species of the *Pteris* genus capable of arsenic hyperaccumulation. *Pteris cretica* and *Pteris longifolia* have been identified as arsenic hyperaccumulators [7]. However, not all species of *Pteris* plants are hyperaccumulators and most of the species can accumulate only low levels of arsenic. The only known arsenic hyperaccumulator that is not part of the *Pteris* species is *Pityrogramma calomelanos* which belongs to the same family Pteridaceae which makes it a fern. Some ferns have evolved in arsenic-rich environments that more advanced species of plants did not and have retained this trait of hyperaccumulating arsenic even after environment became less concentrated in arsenic [8].

Pteris cretica can accumulate more than 1000 mg kg^{-1} of arsenic in its above ground biomass which satisfies the definition of hyperaccumulator [10]. *P. cretica* has been proven to have a similar translocation factor as *P. vittata* but is slightly less efficient in its uptake of arsenic when compared to *P. vittata*. Only *P. cretica* and *P. vittata* show no signs of arsenic toxicity when grown in soils with arsenic concentrations greater than 10 mg kg^{-1} [11]. These two species of ferns are the only hyperaccumulators capable of detoxifying the effects of arsenic well enough to prevent most symptoms from toxicity.

Similar to *P. vittata*, the majority of the arsenic in *P. cretica* can be found in the fronds, opposed to non-hyperaccumulators that store the majority of arsenic in the roots. The transport of arsenic to the fronds is rapid in hyperaccumulators, and short-term unidirectional influx of As(V) into the roots is driven by root concentration gradients [12]. Once *P. cretica* takes up arsenic, it is immediately transported into the shoots which causes the roots to have a lower concentration of the metal. This low concentration of arsenic influences more uptake by the plant. The mechanism that allows the uptake of arsenic into these plants are that their phosphorus transport proteins have a greater affinity for As(V) than non-hyperaccumulating plant species.

The bioaccumulation factor (BF) of *P. cretica* ranges between 1.34 – 6.62 while *P. vittata* exhibits a BF of between 0.06 – 7.43 when based on total arsenic concentrations in the soil [13]. While *P. vittata* has a potentially larger BF, *P. cretica* shows a more consistent level accumulation, suggesting that it might have a more constant rate of arsenic accumulation. So, while *P. cretica* is a lesser known species of

hyperaccumulator, it has the potential to be more consistent in its uptake. *P. cretica* is also a common terrarium plant so its availability is far more widespread than that of *P. vittata*. There are several cultivars of *P. cretica,* and they all exhibit roughly similar levels of arsenic uptake.

2.2. Organic Acids

A strategy many plants employ to increase the bioavailability of nutrients in the soil is to secrete organic acids that promote nutrient release into the soil solution. Mechanisms of enhanced bioavailability include acidification of the rhizosphere, complexation/chelation, and reduction/oxidation. By releasing high levels of acids into the rhizosphere, some plants are capable of decreasing the pH of the rhizosphere and solubilize some nutrients. Low pH also is more conducive for reduction of ferric iron to ferrous iron, the form of iron that is available to most plants. Organic acids also can form complexes with some metals; complexation is the primary mechanism employed to increase the bioavailability of heavy metals [18].

Organic acids exuded by plant roots generally are low molecular weight compounds with one or more carboxyl groups. Depending on the dissociative properties of the carboxyl groups and the number of them, the acids can possess one or more negative charges that act as the site(s) of complexation with soluble metal cations [14]. Generally, acids with a greater number of negatively charged sites (such as citrate and oxalate) are more capable of complexing metals than monocarboxylic acids (such as acetate). In calcareous soils, in which nutrients may be minimally available, many plants employ the strategy of releasing elevated levels of acids to induce the dissolution of

insoluble minerals such as phosphate and ferric minerals, two of the most limiting nutrients in calcareous soils.

Some organic acids, such as citrate and malate, form strong complexes with iron in soil and force the dissolution of some insoluble forms of iron like ferric oxyhydroxides [15]. The ferric-organic complex migrates to the surface of the root where the Fe(III) is reduced to Fe(II), breaking the complex, allowing Fe^{2+} to be assimilated by the root and the organic (e.g., citrate) migrates back into the rhizosphere and starting the cycle again. This strategy is one typically used by many dicots but grasses often employs a different tactic.

In phosphorus deficient environments, plants also produce excess organic acids, again primarily in the forms of citrate and malate, and these complexing agents can solubilize phosphorus from Ca-, Fe-, or Al-P minerals in the soil by complexing the metal and releasing the phosphate. This process can require up to 25% of the plant's photosynthetically fixed carbon but does not seem to significantly impact the production of dry matter in some plants [16]. Unlike malate and citrate, oxalate has the potential to precipitate from solution when in the presence of Ca^{2+}, which reduces its ability to complex other nutrients but has the added benefit of solubilizing phosphorus from calcium containing minerals like apatite. Generally, mobilizing sufficient levels of phosphorus from calcareous soils requires that plants release relatively high concentrations of acids (>100 μM of citrate) [17].

2.2.1. Phytic Acid

An unusual acid that the arsenic hyperaccumulating ferns of the *Pteris* genus produce is phytic acid. Phytate is the principal storage form of organic phosphorus in plants, normally found in the seeds for storage, but it is found in the root exudates of some species of *Pteris* plants [20]. Phytic acid is not commonly observed in any root exudates except for the hyperaccumulating species of ferns. The most predominant forms of low molecular weight acids found in the root exudates of these ferns is phytate and oxalate, but the majority of the organic acid produced is phytate [21]. Exudation of phytate is counterintuitive considering that the plant usually grows in phosphorus deficient conditions. However, phytate is very effective in its chelation of some metals namely calcium, magnesium, and iron [20].

Phytic acid is also much more efficient in solubilizing insoluble forms of phosphorus, such as apatite, than other types of acids. When phytate and oxalate were mixed separately with phosphate rock at different concentrations, the phytic acid released up to 1.5-fold the amount of phosphorus as oxalate [22]. This phenomenon is driven by the number of negative sites that exist on the acid. Phytic acid has six orthophosphate groups which release up to 12 protons giving phytate a very strong ability to chelate calcium [23]. Phytic acid in solutions below pH 8.5 have lost 9 of their 12 protons [82]. After the phytate chelates with calcium, the phosphate that was previously bound to calcium is then released into the soil solution and becomes available for plant uptake.

Phytate is usually unavailable for plant uptake due to its stability and resistance to biochemical degradation, but *Pteris* hyperaccumulators are capable of breaking it

down using the enzyme phytase [24]. Phytase is exuded by other plant species, but the *P. vittata* phytase seems to be more stable and retains its activity for longer periods of time [25]. Phytase enzyme is even more active, up to 4 times higher, when the plant is in a phosphorus deficient environment opposed to an environment with adequate levels of P [25].

Due to *Pteris* arsenic hyperaccumulators often growing in environments high in arsenate, the effect of arsenate on phytic acid secretion plays a role in the plant's ability to uptake the metalloid. Because As(V) is an analog for P, it can interfere with phosphorus uptake and interfere with the metabolism of P in plants [26]. For plants that are arsenic hyperaccumulators, arsenic alone doesn't impact the levels of phytic acid it produces. However, the combination of phosphorus and low levels of arsenic (0.1mM arsenate) can cause the plants to increase production of phytic acid up to 4.9-fold [22]. Arsenate has the opposite effect on the production of oxalic acid in a non-hyperaccumulating fern, in that oxalate exudation drops steeply once appreciable levels of arsenic are in the system. For the hyperaccumulating varieties of ferns, moderate levels of arsenic (<1mM) promote growth [27].

Arsenic binds strongly to iron and forms solid phases in soils, and, because of this, the availability of iron decreases when there are significant levels of arsenic in the soil. As such, due to exacerbated iron deficient conditions, plants may employ the strategy of releasing even more organic acids into the rhizosphere. These organic acids play a role in mobilizing the iron for plant uptake but have added effect of releasing the arsenic that has been bound to the iron [28]. However, there is a large difference in the

efficacy of the organic acids in the solubilization of As and Fe. Phytic acid is the most effective acid at low organic acid: $FeAsO_4$ ratios (1:1 and 2:1) and comparable to citric acid when at high ratio (> 10:1) [22]. Due to the high concentrations of arsenic often found at the contaminated sites, the effectiveness of phytate at low concentration ratios is essential in its effectiveness to mobilize the maximum amount of arsenic for uptake. In fact, under arsenic stress, the plant is stimulated to produce even more phytic acid causing a positive feedback loop. This strategy is not fully understood because increased production of phytic acid releases more arsenic that must be detoxified upon assimilation. The binding affinity of phytic acid also increases exponentially with cation valence which makes it especially effective in chelating the Fe^{3+} in $FeAsO_4$ minerals. The phytic acid can then form solid phase, tetraferric phytate, when present at low concentrations relative to iron [29]. Phytase plays a role in how the plant is able to uptake the insoluble ferric phytate, most likely from decomposing the phytate, thus solubilizing the iron.

2.3. Phosphorus Amendments

Phosphorus is an important plant nutrient and soils often need to be amended with phosphorus fertilizer when the available soil concentrations are below plant requirements. When plants are unable to acquire the phosphorus they need, they often increase their root length and density to fully maximize the contact the plant has with the soil [30].

Adding supplemental phosphorus has a large impact on the mobility of arsenic and affects remediation efforts. The chemistries of arsenate and phosphate are similar,

and the addition of one can affect the chemical interactions of the other. In plants that are arsenic tolerant, arsenic is assimilated by the roots via high affinity phosphate transporters [31]. Arsenic competes with phosphorus for these uptake sites and can compound phosphorus deficiency symptoms. Similarly, phosphate is able to displace arsenate from soil exchange sites, releasing arsenic into solution and increasing its bioavailability for hyperaccumulators. Adding labile phosphorus can increase the plant's ability to remediate an area while providing P nutrition for many growing cycles.

2.3.1. Conventional Fertilizers

Conventional P fertilizers are commonly sourced from phosphate rock, a form of apatite, and then treated to make the phosphorus readily available. Mined phosphate rock can be reacted with sulfuric acid or phosphoric acid to produce soluble phosphate fertilizers. These fertilizers are designed to release their nutrients immediately. Once the fertilizers are added to the soil, the plant is rapidly presented with an abundance of nutrients. Although the surge in soluble P will release As from adsorption sites, the high concentrations of P will overwhelm the arsenic in solution and diminish As uptake. In *Pteris* ferns, increased root growth is associated with the plants growing in environments with sparingly soluble forms of phosphorus [32]. In systems with highly soluble phosphorus, the ferns show decreased root length, shorter root hairs, and decreased rooting density as opposed to using a more insoluble form of phosphorus such as apatite [32]. However, the addition of phosphorus fertilizer has been to shown to increase the arsenic uptake of the plants as opposed to a control that did not receive fertilizers. In an environment with limited amounts of phosphorus and further compounded by the

competition from arsenic, plants didn't thrive well and exhibited the least arsenic uptake, the lowest aboveground and belowground biomass, and didn't survive multiple harvests. Frond biomass increased between harvests when grown in soils treated with rock phosphate or soluble phosphorus [32].

Ground, untreated phosphate rock is a common P fertilizer in acidic soils but is rarely used in alkaline soils. Phosphate rock is sparingly soluble above pH 7 compared to acidic conditions where dissolution is promoted. The P in phosphate rocks comes from poorly crystalline apatite that is accompanied by a variety of impurities [49]. The chemical reactivity and thermal stability can vary widely dependent upon the degree of isomorphic substitution of carbonate for phosphate in the apatite crystal lattice. The greater the carbonate substitution, the higher the solubility. When being considered as a commercial phosphorus fertilizer, manufacturers estimate the reactivity of the phosphate rock, and only samples considered to be "highly reactive" are recommended for use without pretreatment [50].

2.3.2. Apatite

Apatite is the least soluble phosphate mineral in near-neutral and alkaline environments, with the formula $Ca_5(PO_4)_3(OH, F, Cl)$. Apatite can be found as hydroxyapatite, fluorapatite, and chlorapatite; fluorapatite is the most common in nature and the most stable apatite species. Being stable in alkaline soil, little bioavailable phosphate is present for plants without outside influences.

The stability and relative insolubility of apatite results from its highly ordered crystal structure. Crystals of the calcium apatite species consists of individual

orthophosphate tetrahedra that is linked by calcium cations [33]. The first calcium site is occupied by four calcium atoms and has a coordination number of nine, the second calcium site is occupied by six calcium atoms and has a coordination number of seven [33]. Fluoride, hydroxide, and chlorine are located within the channels of the second calcium site and have high mobility. As pH increases, the solubility of calcium phosphate minerals decreases; the solubilities of iron phosphates (strengite) and aluminum phosphates (variscite, wavellite) increase with increasing pH. Phosphate eventually precipitates as fluorapatite in alkaline soils except in leached soils where the levels of calcium activity and pH do not meet the requirements for its formation [34].

Apatite is the most effective source of phosphorus for maximizing arsenic uptake by hyperaccumulating plants. While most plants cannot acquire sufficient quantities of P from apatite in alkaline soils, concentrations of P in the roots of *P. vittata* amended with apatite increased by 49% and increased by 28% in the fronds compared to P concentrations in plants grown in soils with no amendments [22]. Low soluble phosphorus conditions in soils promote increased arsenic uptake and have been associated with greater root growth [35]. These observations can be attributed to the fact that most arsenic hyperaccumulating plants evolved in conditions that are relatively low in phosphorus and high in arsenic. The overall aboveground biomass has been observed to be higher under conditions of low phosphorus but high arsenic conditions [78]. Plants that did not evolve to tolerate arsenic usually show lower growth in soils high in arsenic [74]. The *P. vittata* plants have adapted to these conditions and respond best to conditions that mimic their evolutionary background. So, the use of a mildly soluble

source of phosphorus is essential to maximizing the As-phytoextraction prowess of these plants.

Increased root density and the greater number of root hairs allow the plant to have more contact with the surrounding soil and effectively increase the volume of the rhizosphere. The plant will be more metabolically efficient in acquiring nutrients due to its high surface area to volume ratio [37]. A larger root system also produces more root exudates which has the added benefit of mobilizing both phosphorus and arsenic. Because apatite is sparingly soluble, it can maintain low phosphorus conditions but still provide adequate P to the ferns throughout the entire growing period when solubilized by organic acid exudation. If levels of phosphorus are too high, arsenic and phosphorus can exert antagonistic effects on each other when it comes to plant uptake and transport, resulting in overall lower levels of arsenic uptake [36].

2.4. Metal(loid) Reactions

Metals ions are retained in the soil by sorption, precipitation, or complexation with the soil matrix. In sorption reactions, charged species in the soil solution (ions) are attracted to charged soil surfaces through electrostatic attraction and through the formation of specific bonds [40]. Complexation is the association between two or more molecules: the association can be covalent (e.g., coordination complex) or ionic bonds (inner sphere complexes) or relatively weak forces such as van der Waals forces (outer sphere complexes), hydrogen bonding, and hydrophobic interactions [41]. Complexation reactions in soil often involve organic matter dues to its strong affinity for metal cations through ligands that interact with metals. With increasing pH, the acidic functional

groups deprotonate and thereby increase the affinity of ligand ions for organic matter. Precipitation is the main process of metal immobilization in soils with high pH when there are anions present [42]. Species of metalloids that form anionic molecules, such as arsenic, have the potential to precipitate from solution when bonded to certain metal cations such as Ca^{2+} and Fe^{3+}. Precipitation of metal phosphates is considered the primary mechanism for phosphorus induced immobilization of metals, especially in substrates containing large concentrations of metals [38]. Phosphorus compounds can act as both a source and a sink of for heavy metals in soils.

2.4.1. Arsenic Mobilization

Phosphorus influences arsenic mobility and bioavailability, the degree of which depends upon the specific components of the soil binding the arsenic. Phosphate commonly has been used to immobilize some metal(loid)s including lead and zinc but has the opposite effect on arsenic [38]. This can be used to the advantage of those who wish to phytoremediate an area. Arsenic is mobilized when soluble P is added to the system because phosphate ions are more strongly sorbed onto soils exchange sites than As ion, thus displacing As into the aqueous phase [39]. The bioavailability of the arsenic increases, and the plants are able to acquire the As via the phosphate transporter system.

Arsenate and phosphate are chemically similar anions that compete for the same mineral surface sites. Primarily, the desorption of arsenate is caused by ligand exchange in which $H_2PO_4^-$ displaces $H_2AsO_4^-$ from the adsorption sites. The strong attraction of phosphate for positively charged sites at mineral surfaces results in displacement of ligands such as $-OH^-$ and $-OH_2$ on the mineral edges [43]. This ligand exchange

reaction results in the phosphate ion bonding to the coordination sphere of the metal ion at the mineral's edge. Phosphate has strong bonding capabilities with metals and is capable of forming stable minerals because of the chemistry of phosphorus and oxygen. The electronegativity of phosphorus is appreciably less than that of oxygen, so the electron distribution and negative charge is spatially associated with the surface of the oxygen tetrahedra. In effect, metals with residual positive charge at the edges of minerals (such as metal oxides and aluminosilicates) constitute sites of strong electrostatic attraction for the residual negative charge on the surface of the phosphate ion [44]. However, the effect of $H_2PO_4^-$ on metal desorption depends on the nature of the soil, its sorption capacity, and the extent of saturation with a particular metal ion. Soils high in variable charge minerals (Al or Fe oxides, allophanes) do not release arsenate as easily. Large additions of phosphate are needed to mobilize arsenate in soils that have a high anion fixing capacity. In soils that are high in allophanes, which exhibit a high anion fixing capacity, the sorption of phosphate would be enhanced but the release of already bound arsenate would inhibited [46].

Competitive sorption of phosphate and arsenate can be impacted significantly by soil mineralogy. Kaolinite, gibbsite, boehmite, allophane, and non-crystalline aluminum hydroxides exhibit a much greater affinity for phosphate than arsenate. The opposite is true for goethite, pyrolusite, birnessite, nontronite, ferruginous smectite, and ferrihydrite. Montmorillonite, vermiculite, and illite showed a slight preference for phosphate over arsenate [45]. The competitiveness of the anions also changes at different pH values. In

soils with a neutral or alkaline pH, phosphate inhibited arsenate sorption on the surfaces of soil particles more than in acidic conditions.

2.4.2. Lead Immobilization

The primary method of *in situ* immobilization of Pb in soils is the reaction of lead with phosphate thus forming insoluble pyromorphites [$Pb_5(PO_4)_3(F, OH, Cl)_2$]. Pyromorphite is stable and sparingly soluble under normal soil conditions and is expected to form in Pb-contaminated soils if sufficient phosphorus is available [47]. With adequate levels of both lead and phosphorus, the formation of the new mineral is rapid. The mass ratio of Pb:P:Cl in pyromorphite is 23:3:1 so relatively small quantities of phosphorus and minor quantities of chloride are necessary for this mineral to form [48].

Phosphorus amendments are necessary when trying to immobilize Pb in contaminated soils due to inadequate levels of soluble phosphorus present in natural systems. Apatite can immobilize Pb in soils when in the presence of anions or cations. The primary mechanism is the dissolution of the apatite and the release of phosphate ions into the soil solution. XRD analysis on soils treated with apatite have shown peaks that could be identified as fluoropyromorphite-like minerals [49]. This contributes to the conclusion that apatite dissolution, primarily existing in the form of fluorapatite, followed by fluoropyromorphite precipitation is the primary mechanism for lead immobilization.

The stability of pyromorphite in soil can be attributed to its crystal structure. The atomic arrangement of pyromorphite is an apatite isostructure [51]. In the structure Pb

binds to six oxygen atoms in the form of a trigonal prism that has three longer bonds to oxygen atoms through the prism face. Not only aqueous Pb^{2+} can contribute to the formation of pyromorphite species. Adsorbed Pb, Pb-bearing minerals, and Pb organic compounds must be considered because they represent the main sources and sinks for soil Pb and are the primary factors that determine the availability of free Pb ions in the soil solution [53]. The adsorption/desorption of Pb is pH dependent and occurs predominantly under acidic conditions. However, phosphate anions can interact with adsorbed Pb on iron oxide surfaces to precipitate pyromorphite like minerals on the surfaces of iron oxides [72]. The precipitation of pyromorphite is a rapid process, taking only seconds, while the adsorption of phosphorous onto iron oxides is relatively slow [73]. Therefore, phosphate ions are more likely to interact with Pb ions rather than the surfaces of minerals to form sorbed complexes [72].

To test the stability of the pyromorphite species that form after reactions with apatite, samples containing the mineral species were leached at pH values ranging from 3 to 12. After leaching, the samples were analyzed using XRD and discovered no differences in peak intensity signifying that the newly formed mineral is stable under a range of pH values [52]. Other forms of lead phosphates have not been observed to precipitate out due to the low concentrations of lead and phosphorus in the soil solution. Lead has also been found to precipitate or adsorb onto the surface of apatite [53].

2.5. Objectives and Hypothesis

The overarching objective of this research is to test the impacts of apatite additions to contaminated soil on the bioaccessibility of arsenic and lead as well as arsenic uptake by an arsenic hyperaccumulator. Subobjectives include:

1. Determine the effect of apatite particle size and P:As molar ratio on arsenic uptake by *Pteris cretica* as well as the bioaccessibility of As and Pb determined by the IVBA test.
2. Evaluate the ability of *Pteris cretica* to solubilize apatite in the soil, thus increasing the bioavailability of arsenic.

The supporting hypotheses are:

1. Lead bioaccessibility will decrease with decreasing apatite particle size, and arsenic bioaccessibility will increase with decreasing apatite particle size,
2. Additions of apatite will significantly increase the plant uptake of arsenic.
3. Arsenic uptake by *Pteris cretica* will be correlated to $NaHCO_3$ extractable arsenic.

3. PHYTOREMEDIATION OF ARSENIC CONTAMINATED SOIL USING *PTERIS CRETICA* AND APATITE AMENDMENTS

3.1. Introduction

The soil used for this research was sampled from the Jacob Smelter superfund site located near Stockton, Utah, which covers about eight square miles. The source of the contamination was mining and smelting of gold, silver, copper, lead, and zinc that occurred primarily from the 1860s through the 1870s [57]. A total of nine different smelters existed in this area during this time, all contributing to the contamination. Throughout this period, waste was generated in the form of heavy-metal contaminated soils, mill tailings, and smelter wastes [58].

The average concentration of the contamination at this site is 66,400 mg Pb kg^{-1} and 7,520 mg As kg^{-1}. The majority of the contamination is limited to the upper 45 cm of the soil [58]. Based on US EPA standard protocols for assessing risk of cancer and non- cancerous diseases, an action level of 500 mg kg^{-1} Pb and 100 mg kg^{-1} As was used for residential surface soil; 800 mg kg^{-1} Pb for residential subsurface soil; 3000 mg kg^{-1} in both surface and subsurface soil for recreational areas; 2,200 mg kg^{-1} of Pb for surface and subsurface soils in for commercial and industrial areas [58]. To achieve these levels, the record of decision filed by the EPA recommends excavating the contaminated soil to a maximum of 45 cm and placing the soil in an offsite land-fill. The projected time is 12 months at a cost of $9,647,000.

Phytoremediation is an emerging field proven to be an option to excavation for the remediation of moderately polluted sites [4]. The approach takes advantage of plants

capable of accumulating large quantities of arsenic above ground. Several *Pteris* species are known hyperaccumulators of arsenic in soils, but the most well know is *Pteris vittata*. This species has the potential to absorb high concentrations of arsenic, up to 2% dry weight mass, without displaying symptoms of arsenic toxicity [1].

Arsenic is non-essential and generally toxic to most plants. Elevated soil concentrations inhibit root extension and proliferation [54]. Upon translocation to the shoot, arsenic can severely limit plant growth by slowing or even stopping expansion and biomass accumulation, as well as compromising plant reproductive capacity through losses in fertility, yield, and fruit production [75]. At sufficiently high concentrations, arsenic interferes with critical metabolic processes, which can lead to death [54]. Most plants possess mechanisms that restrict the accumulation of arsenic to only the roots. However, species of hyperaccumulating ferns are capable of handling high levels of arsenic because of unusual mechanisms to reduce the toxicity of the absorbed metal [36]. Rather than minimizing As toxicity through exclusion, *Pteris vittata* readily assimilates both As(III) and As(V), reduces As(V) to As(III), and transports the As(III) to the fronds [2].

A relatively new species of hyperaccumulator, *Pteris cretica* can accumulate over 1000 mg kg^{-1} of arsenic in its above ground biomass, which satisfies the definition of hyperaccumulator [10]. *P. cretica* has a similar translocation factor as *P. vittata* but is slightly less efficient in As uptake. Only *P. cretica* and *P. vittata* show no signs of arsenic toxicity when grown in soils with arsenic concentrations of greater than 10 mg

kg^{-1} [11] because these two species of ferns are the only hyperaccumulators capable of detoxifying arsenic and preventing toxicity symptoms.

The transport of arsenic to the fronds is rapid in hyperaccumulators, and short-term unidirectional influx of As(V) into the roots is driven by concentration gradients [12]. Immediately after As uptake, *P. cretica* transfers the As from the roots to the shoots, lowering the root As concentration and restoring the concentration gradient. The transport proteins in hyperaccumulating plants that are normally responsible for P(V) uptake have a greater affinity for As(V) than the transport proteins in non-hyperaccumulating species [76].

In some environments, nutrients may not be readily available, so a strategy many plants employ to increase the bioavailability of nutrients in the soil is to secrete organic acids that promote nutrient release into the soil solution [14]. Organic acids release nutrients through acidification of the rhizosphere, complexation/chelation, and reduction/oxidation, all of which have some roll to play in nutrient acquisition. By releasing high levels of acids into the rhizosphere, some plants are capable of decreasing the pH of the rhizosphere [67]. This has the benefit of solubilizing some nutrients while also creating an environment that is more conducive for reduction of ferric iron to ferrous iron, the form of iron that is more available for many species of plants [77]. Organic acids can also form complexes with some metals effectively chelating the metal for plant uptake. Complexation is the primary mechanism employed to increase the bioavailability for most heavy metals [18].

Phytate is the principal storage form of organic phosphorus in plants, normally found in the seeds, but it is found in the root exudates of some species of *Pteris* plants [20, 68]. The most predominant forms of low molecular weight acids found in the root exudates of these ferns are phytate and oxalate, but the majority of the organic acid produced is phytate [21]. However, other studies have seen the opposite in which the most prominent acid exuded by the hyperaccumulating *Pteris* ferns is oxalate [68]. Exudation of phytate in plants is a reasonable adaptation considering that the plant usually grows in alkaline, phosphorus deficient soil conditions. In soils, phytate is very effective in its chelation of calcium, magnesium, and iron [20]. This has the benefit of allowing the plant to absorb more nutrients besides phosphorus.

Phytic acid is also much more efficient in solubilizing insoluble forms of phosphorus, such as apatite, than other types of acids [24]. When phytate and oxalate were mixed separately with phosphate rock at different concentrations, the phytic acid released as much as 1.5-fold more phosphorus than oxalate was capable of releasing [22]. Phytic acid has six orthophosphate groups which have the ability to release protons into the system, with some acidic sites immediately deprotonating while others do not their protons until high pH. [23]. After the phytate chelates with calcium, the phosphate that was previously bound to calcium is then released into the soil solution available for plant uptake.

Soil microorganisms assist in the solubilization of the insoluble sources of phosphorus as well as the recycling of the phytate in the plant exudates. Seven arsenic-resistant bacteria have been identified in the rhizosphere of *P. vittata* that have

developed the capacity to survive in the high-As conditions. These effectively release phosphorus from insoluble inorganic sources and from organic sources such as extracting phosphorus from phytate [70]. Five of the 7 species were more effective in solubilizing phosphorus than non-resistant bacteria and employed both enzymes and siderophores to do so [71]. The bacteria seem to be specialized in dissolving a specific form of phosphorus: those effective in solubilizing phytate are less efficient in dissolving phosphate rock and vice versa.

When some plant species are unable to acquire the phosphorus they need, they increase their root length and density to fully maximize the contact the plant has with the soil [30]. Adding supplemental phosphorus has a large impact on the mobility of arsenic and affects remediation efforts. Due to the very similar chemistries of arsenate and phosphate, the addition of one can affect the chemistry of the other. In plants that are arsenic tolerant, arsenic is assimilated into the root via high affinity phosphate transporters [31]. Arsenic, thus, can compete with phosphorus for uptake and can further compound phosphorus deficiency symptoms. Phosphate is able to replace arsenate on soil sorption sites, releasing arsenic into solution, and increasing its bioavailability for hyperaccumulators. Adding phosphorus can increase the plant's ability to remediate an area while providing the nutrition for many growing cycles.

Apatite is an effective source of phosphorus for encouraging arsenic hyperaccumulating plants to acquire the highest concentrations of the metalloid. While most plants cannot solubilize sufficient quantities of phosphate from apatite, phosphorus concentrations in the roots of *Pteris spp.* grown in soils amended with apatite increased

by 49% and P concentrations in the fronds increased by 28%compared to plants grown in soils with no P amendments [22]. Low soluble phosphorus conditions in soils promoted increased arsenic uptake as well as greater root growth [35]. These symptoms can be attributed to the fact that most arsenic hyperaccumulating plants evolved in conditions that are relatively low in phosphorus and high in arsenic. In addition to increased root growth, the overall above ground biomass increases when grown in low phosphorus but high arsenic conditions [78]. This is the opposite from plants that did not evolve to tolerate arsenic which usually show lower growth when in soils high in arsenic. The hyperaccumulating plants have adapted to these conditions and respond best to conditions that mimic their evolutionary environment. The use of a mildly soluble source of phosphorus is essential to maximizing the phytoremediation prowess of these plants.

Chemical extractants often are used to predict the portion of the contaminants that are available to target organisms. Direct ingestion is the most important human pathway of exposure to Pb from contaminated soils and is a key pathway for As. The biological availability of As and Pb from ingested soil can be evaluated through the In Vitro Bioaccessibility Assay (IVBA). The concentrations of Pb and As extracted by this technique are correlated to As and Pb found in pigs if they ingested contaminated soil. Because the swine digestive system is an excellent model for human, this has then been extrapolated to humans and used as a measure of the toxicity of soils [63]. The chemistry of IVBA mimics the conditions of the human stomach, and the metals extracted should have a 1:1 correspondence to the levels of metals assimilated if a

human happens to digest the soil. Because the area from which the soil was collected is located next to a residential area, characterization the potential effects of accidental ingestion and inhalation of the contaminated soil is relevant.

The Olsen method for extractable P is used to gauge the concentration of inorganic orthophosphate that is bioavailable to the plant in neutral to alkaline soils [70]. The sodium bicarbonate extraction was specifically developed to correlate with the plant availability of phosphorus in alkaline (particularly calcareous) soils. While originally adapted as an index of plant bioavailable phosphorus, the method was found to also correlate with plant available arsenic [80].

Due to the high levels of metallic contamination of this soil, an *in situ* remediation method would be useful that could immobilize both As and Pb. The antagonistic remediation chemistries of the two contaminants makes simultaneous immobilization of both contaminants very challenging. Immobilizing lead using phosphorus has the effect of mobilizing arsenic in the soil, which is contrary to the concept of in situ immobilization. However, P-induced mobilization can be used as an advantage if using a plant that can hyperaccumulate arsenic. In soils with extreme contamination, fixing Pb as pyromorphite with soluble P will require quantities that would overwhelm the plants with salts and other side reactions. However, if the source of phosphorus is sparingly soluble, like apatite in alkaline soils, the *Pteris spp.* are able to slowly solubilize the mineral releasing a low amount of phosphorus into the system. This then allows the newly liberated phosphate to bind with the Pb and also replace As on soil exchange sites which makes the metalloid more available. Particle size and rate

of phosphorus additions are being investigated to determine the most optimum strategy for the remediation of the contaminants. The goal of this research is to test the impacts of apatite additions to contaminated soil on the bioaccessibility of arsenic and lead as well as arsenic uptake by an arsenic hyperaccumulator

3.2. Materials and Methods

3.2.1. Soil Characterization

The surface layer (<20 cm) of a soil highly contaminated with As and Pb was collected from a former smelting site in Stockton, Utah. The soil belongs to the Abela series: deep, well-drained Typic Calcixerolls formed from alluvial and lacustrine deposits. The Abela soils are derived from limestone, sandstone, and quartzite parent material. The typical textural class is clay loam with 25% sand, 38% silt, and 37% clay. The soil sample was air dried and sieved (<2 mm) prior to use. Soil pH, electrical conductivity, organic matter content, total metal content, and particle size were determined using the standard protocols. The contaminated soil was tested to determine its hazardous waste disposal status using Toxicity Characteristic Leaching Procedure [47].

The Ozona series, located in Ozona, Texas, was the series that most closely resembled the contaminated soil in Texas and was used to make all dilutions. The Ozona series are loamy, mixed, superactive, thermic, shallow Petrocalcic Calciustolls. These well drained, moderately permeable soils formed in calcareous loamy eolian sediments overlying marl and limestone of the Cretaceous-age Buda Limestone Formation. These

nearly level to gently sloping soils are on plain surfaces of the Edwards Plateau. Slopes range from 0 to 3 percent. Both of the soils are clay loams, calcareous, have mixed minerology, and have an organic matter content around 3%.

The total Pb (66,400 mg Pb kg^{-1}) and total As (7,520 mg As kg^{-1}) concentrations in the original soil are at the high end of the spectrum for contaminated soils. These concentrations would have presented a number of practical challenges in this research project, particularly the requirement for extreme concentrations of amendments. Therefore, the soil was diluted to two different concentrations to make the metal concentrations more manageable: a) 1-part contaminated soil mixed with 4-parts uncontaminated soil for a 1:5 dilution (mass:mass) and b) 1-part contaminated soil mixed with 9-parts uncontaminated soil to make a dilution of 1:10 (mass:mass).

Table 3-1: Basic soil properties of the contaminated Abela soil and the uncontaminated Ozona soil.

Soil Series	Abela	Ozona
pH	8	7.8
EC (dS m^{-1})	0.13	0.26
Texture	Clay loam	Clay loam
clay (%)	37	37
silt (%)	38	38
sand (%)	25	25
Organic Matter (%, w/w)[2]	3.4	3.02
CEC (cmol kg^{-1})[3]	20	21
Phosphorus (mg kg^{-1})[4]	23	17
Calcium (mg kg^{-1})[4]	3700	16,768
Magnesium (mg kg^{-1})[4]	735	189

[1]Determined by hydrometer method; [2]Mass loss by ignition; [3]$BaCl_2$ method; [4]Mehlich(III) extraction

The plant available concentrations of phosphorus are too low to sustain rangeland grasses without the plants showing symptoms of phosphorus deficiencies in both of the soils. For range grasses, the Ozona soil would require 122 lbs. P_2O_5/acre to meet the demand of the plants for phosphorus and the Abela soil would require 105 lbs. P_2O_5/acre to satisfy optimum plant growth. The P fertility status of both soils are classified as "Low."

3.2.2. Phosphorus Amendments

The soil was amended with crystalline apatite to test the impact of particle size and P:As ratio on a) the assimilation of As and Pb by the plants; b) bioaccessible As and Pb as determined by the In Vitro Bioaccessibility Assay (IVBA); and c) bicarbonate extractable As and Pb. In previously published studies utilizing an insoluble form of phosphorus, commercially available phosphate rock often was the preferred P source. However, phosphate rock generally contains poorly ordered apatite with some degree of carbonate substitution and tends to be more soluble than pure crystalline apatite. Commercial manufacturers also sell phosphate rock that is "reactive" so its chemistry differs from pure apatite.

To control the size of the particles and ensure that the source of phosphorus added was uniform, the soil was amended with a crystalline apatite (source). Using large apatite rocks sourced from Madagascar, the apatite was ground into three particle sizes that were expected to have significantly different dissolution rates. The apatite was ground to 500-250 μm, 250-105 μm, and <105 μm. This was achieved by breaking up the apatite rocks into more manageable pieces before grinding the rock fragments using a porcelain mortar and pestle prior to sieving. The ground apatite was sieved through three screens with different mesh sizes that would separate the apatite particles into the desired sizes.

The most finely ground apatite was analyzed for total P using x-ray fluorescence (DP-6000 Delta Premium, featuring Rh X-ray tube) and found 12% P.

Using the analytical values of P in the apatite, P was added to the contaminated soils at concentrations expected to impact the rates of arsenic mobilization, plant uptake, and (less likely) Pb immobilization. The phosphorus was added in P:As molar ratios of 1:1, 1:2, and 1:5. With previous analysis of the soils showing the concentration of arsenic at 7520 mg kg^{-1} the concentrations in the soils after dilutions would be about 752 mg kg^{-1} for and 1504 mg kg^{-1} at the 1:10 and 1:5 soil dilutions, respectively. So, at the greatest concentrations of arsenic and the highest ratios of P:As, 33.84 grams of apatite was added to the soil. The lowest amount of apatite added was 406 mg for the treatments that involve the greater dilution of contaminated soils and the smallest ratios of P: As. The three particle sizes were used for each of the three ratios giving 9 different treatments. Plants also were grown with zero phosphorus amendments (control) for a total of 10 different treatments for each soil dilution. All treatments were established in triplicate for a total of 60 experimental units.

3.2.3. *Pteris cretica*

Although *P. vittata* is the species most often grown in these types of experiments, it is not available commercially. Spores of the plant are usually only available from other researchers studying the species or propagated in few nurseries in the spring. Therefore, *P. cretica* was chosen because of its availability and similar characteristics to the more well-known *P. vittata*.

Seventy-two *P. cretica* plants, at a few weeks old, were ordered from a terrarium supplier (Black Jungle Terrarium Supply, Greenfield, MA) and, upon receipt, immediately replanted in one liter of growing medium composed of peat moss and

perlite. The plants were watered every other day in a greenhouse environment for three months until they all had propagated several new fronds and deemed large enough to be transplanted into the contaminated soil.

3.2.4. Preparation of Amended Contaminated Soil and Planting

The contaminated soil was diluted in two ratios to make the concentrations of arsenic and lead more manageable for a greenhouse experiment. The greenhouse pots were 8 inches in diameter and held about 2 liters of soil weighing (2.7 kg). For the contaminated soil that was diluted at a 1:10 ratio, 2455 grams of Ozona series soil, was added to 270 g Abela series soil. For the 1:5 dilution of the contaminated soil, 2170 g of Ozona soil was added to 545 g Abela soil. Soil for every pot was mixed separately in a sealed container by shaking and rolling to ensure proper homogenization of the sample.

Certain precautions were needed when adding apatite to ensure that the mixture would be homogenous despite the different densities of the substances. Samples of ground apatite were weighed according the treatment. The larger two sizes of apatite, 500-250 μm and 250-105 μm, were put into salt shakers and evenly sprinkled onto the surface of the soil. For <105 μm particle size, the samples were placed in a fine wire mesh and dusted over the soil before mixing. Every pot was amended with apatite individually and mixed in a sealed container to ensure proper homogenization. The container was carefully rolled side over side to ensure even mixing despite the different densities. The sample was not shaken as a measure to prevent the denser apatite from setting towards the side or bottom of the container. After being properly mixed, the soil mixture was carefully transferred to a pot labeled with the dilution and treatment.

Based on prior experience, we were concerned that the plant roots might not emerge beyond the confines of their original peat growing mix because they might be averse to exploring the harsher environment of the arsenic contaminated soil. To counteract this potential problem, the plant roots were washed and massaged thoroughly in distilled water in an effort to remove as much peat as possible from their roots. To minimize damage, the roots were first soaked in the water for a couple of minutes to loosen the soil as much as possible; the roots were carefully agitated. After the majority of the peat was removed from the roots, the plants were placed in a hole in the mixed contaminated soil and covered.

Due to the clayey nature of the soil, the initial watering needed to be closely monitored to ensure that the soil was properly saturated before a more regular watering scheme could be established. The high percentages of clay restricted the percolation of water into the soil. A seemingly saturated soil would then dry and crack after a few hours. This negatively impacts the health of the plant, so the soil was watered every few hours after the top of the soil started to dry. Once the soil no longer cracked after a few hours, a normal watering schedule (every other day) was established taking care not to oversaturate the soil.

3.2.5. Harvesting Plants and Soils

The plants were grown for three months in a greenhouse. Once the plants had matured and exhibited significant new frond growth, the above ground biomass was harvested. All the fronds were trimmed 1 cm above the soil, and the plants were dipped in water to wash off any soil that might have been clinging to them. Due to the high

levels of arsenic and lead in the soils, even trace amounts of the soil clinging to the fronds could skew the concentrations of the metals. Immediately after harvesting, the ferns were transferred to brown paper bags, weighed, and dried for 48 hours at 65 °C, then weighed again for the dry weight.

After the fronds were removed, the soil was harvested. The roots did not consistently penetrate the entirety of the soil and, for the most part, the roots were restricted to the upper half of the soil. Because of this, two separate samples were taken from each pot. A bulk sample of the soil was collected by removing the bottom half the soil profile, about 2 inches below the last traces of roots. Rhizosphere soil was sampled by slightly moistening the soil around the roots and scraping away the soil with a metal spatula. Any roots that detached were removed. Once the soil was collected, it was dried on butcher paper in the greenhouse and placed in labeled plastic bags.

The dried soil and plant samples were ground to <2 mm. The soil was put into a mechanical flail soil grinder, passed through a 2 mm sieve, and returned to its original bag. The plants were ground in a Wiley mill and passed through a 2 mm sieve. The grinder was thoroughly cleaned between samples.

3.2.6. Parallel, Unplanted Greenhouse Experiment

A parallel experiment in the absence of plants was conducted to eliminate the effect of the *P. cretica* on observed changes in the soil chemistry. The experiment was conducted using the same treatments and dilutions but with less soil. Using 118 mL specimen cups, for the 1:10 dilution, 45 grams of Ozona series soil and 5 grams of the contaminated Abela series soil were mixed. In the 1:5 dilution, 40 grams of Ozona series

and 10 grams of Abela series soil were used. Phosphorus was added to the samples at the same ratios and particle sizes as the planted experiment with the maximum amount of apatite being 0.600 grams and the lowest amount being .060 grams of apatite.

Once the soil and phosphorus amendments were added, the sample containers capped and rolled side to side and top over bottom, in the same manner used to mix the soil in the planted version of the experiment. The soil was wetted to field capacity, roughly 34% moisture, and capped. To prevent the sample from becoming anaerobic a small hole was punched into the lip of the specimen cup to allow air flow into the cup. The samples were transferred to the same greenhouse as the plants, and every other week each specimen cup was weighed for water loss and replenished back to field capacity. After 3 months of incubating in the greenhouse, the soil was dried inside of the sample cups and ground to pass a 2 mm sieve using a porcelain mortar and pestle.

3.2.7. Chemical Analyses

Total concentrations of As and Pb for the soil and plant samples were determined using a nitric acid and hydrogen peroxide digest. The soil samples, both bulk and rhizosphere samples, were extracted in additional ways to consider the concentration of arsenic and lead that were available to humans and plants. The In Vitro Bioaccessibility Assay (IVBA) and sodium bicarbonate extraction were used to quantify the concentrations of arsenic and lead available to humans and plants, respectively.

3.2.7.1. Total Digest

Soil samples and the plant samples were digested using EPA method 3050B [64]. Using 1.000 gram of ground and sieved soil or 0.500 grams of plant tissue, combine with

10 mL of concentrated nitric acid in a digestion tube and cap with a vapor retention device to maintain the levels of nitric acid inside of the vessel. The samples were heated to 95 °C in a digestion block and allow to react for 10-15 minutes. The samples were removed from the block to cool to room temperature and an additional 5 mL of nitric acid was added. The samples were heated back to 95 °C and digested for 2 hours. Once the samples had finished heating, the vapor retention device was removed, and they were cooled back to room temperature and a mixture of 3 mL 30% hydrogen peroxide and 2 mL water was added to the samples to finish the process of oxidizing the organic matter in the samples. The samples were heated back to 95 °C to enhance the oxidization of the organic matter. The samples were heated for as long as the hydrogen peroxide was reacting which is exhibited by small bubbles being released. After the activity had stopped, the digestion tubes were cooled to room temperature. Further additions of hydrogen peroxide, 1 mL at a time but no more than a total of 10 mL, were added to the sample and heated and cooled until the addition of hydrogen peroxide no longer caused a reaction in the samples. At this point the samples were reheated to 95 °C and incubated again for another two hours, cooled, and diluted to 75 mL

3.2.7.2. In Vitro Bioaccessibly Assay

The IVBA extraction fluid is comprised of a 0.4 *M* glycine solution that has been acidified to a pH of 2.5 using concentrated hydrochloric acid and heated to 37°C. About 1.000 g of the soil was added to a HPDE plastic bottle and filled with 100 mL of the extracting solution (heated to 37 °C). The sample was shaken 30 rpm on an orbital shaker for 1 h in an incubator at 37 °C. Once the extract was complete, the samples were

allowed to settle for an hour and the aliquots were taken from middle of the extract. Every treatment group had one sample analyzed in duplicate (33% replication) and a blank was present for every 10 samples extracted.

3.2.7.3. Sodium Bicarbonate Extraction

The extractant is 0.5 *M* sodium bicarbonate solution that has been adjusted to a pH 8.5 using a 1*M* sodium hydroxide solution. To extract the soil, 1.000 gram of soil was added to a 50 mL centrifuge tube and filled with 20 mL of the extracting solution. The centrifuge tube was shaken on a reciprocal shaker for 30 minutes at 100 rpm and centrifuged at 2000 rpm for 5 minutes to compact the soil to the bottle of the centrifuge tube. Every treatment group had one sample analyzed in duplicate (33% replication) and a blank was present for every 6 samples extracted.

3.2.8. ICP-MS Analysis

Metal concentrations (As and Pb) in all extracts and digests were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, PerkinElmer NexION 300D). The samples were introduced to the instrument using an autosampler, nebulized into an aersol, and atomized using an argon plasma. Indium was used as an internal standard to compensate for any residual matrix effects and instrumental drift. The detection limit for this instrument for As and Pb is approximately 200 ng/L. Calibration curves were determined from external standards prepared using certified stock solutions (arsenic and lead plasma standard solution from Alfa Aesar) and prepared using reagent grade nitric acid. Sample blanks were analyzed to determine and potentially correct for background contamination.

3.2.9. Phosphorus Analysis

Due to interferences caused by polyatomic ions, ICP-MS is unable to accurately quantify P concentrations without significant modification to the instrument. Therefore, the concentrations of phosphorus in the bicarbonate extracts were analyzed colorimetrically. The most common procedure is the molybdate blue method, based on the reduction of a phosphomolybdate complex to molybdenum blue under acid conditions [61]. However, high concentrations of arsenate in the samples gives a positive interference with P analysis. To negate the arsenate interferences, the arsenate must first be reduced to arsenite by reacting the extracted samples with L-cysteine [62].

The first step is the reduction of arsenate in the solution by adding 300 μL of the (5/5 w/v) L-cysteine solution was added to 10 mL of each bicarbonate extract sample. (Extracts often needed predilution with the extraction solution to place the soluble P in the proper concentration range.) The samples with L-cysteine were heated to 80 °C in a forced air oven and maintained at 80 °C for 5 minutes. The samples were cooled to room temperature, and 500 μL of the ascorbic acid solution was added to the samples. 1.5 mL of acetone and 2 mL of the "mixed reagent" (for color development) were added to the sample and allowed to vent gas before capping and mixing. The samples were allowed to react for 15 min to develop color, and the absorbance was measured at 925 nm using a spectrophotometer (Thermo Scientific, Spectronic 200) [69].

3.2.10. Statistical Analyses

The design of the greenhouse experiment was a 4-way completely randomized with two dilution rates (1:10 and 1:5); three rates of phosphorus applied in P:As molar

ratios (1:1 1:2 and 1:5); mesh sizes (250-500 μm; 105-250 μm, and <105 μm); and planted/unplanted soils. Each treatment, repeated in triplicate, was composed of a dilution, a level of phosphorus addition, apatite particle size, and either planted or unplanted. In all, there were 120 experimental units in this trial. Using the statistical software CoStat version 6.45 (COHORT Software 2019), each extraction was analyzed using ANOVA with an alpha value of 0.05.

3.3. Results and Discussion

Due to inconsistences in the contaminated soil samples, the soil dilution of 1:5 had to be dropped from the experiment because of very different concentrations of arsenic and lead found between bulk soil samples in the dilution series. Although all samples were analyzed (see Appendix), only the results from the 1:10 dilution are discussed.

3.3.1. Plants

3.3.1.1. Biomass as Affected by Treatment

After the plants were harvested, dried, and weighed, average biomass ranged from 3.2 to 6.7 g/pot. Plant biomass responded differently to the treatments of particle size and rate of apatite application (Fig. 3-1).

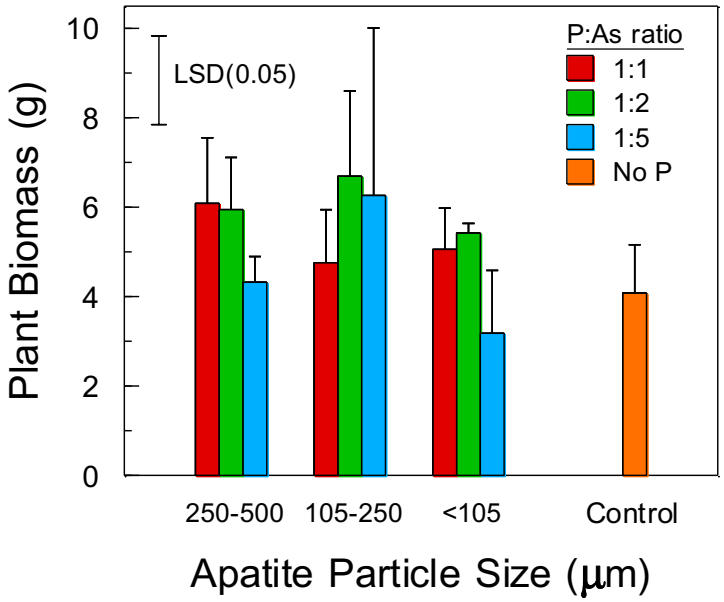

Figure 3-1: Dry biomass of the *P. cretica* as affected by P application rate and particle size.

The 1:2 molar ratio of P:As consistently had high biomass, and the finer particle size (<105 µm) had consistently low biomass. The treatment that produced the greatest biomass was apatite ground to 105-250 µm particle size and added at a 1:2 ratio. Even though 105-250 µm is not the smallest particle size used in this trial, it was more effective in stimulating plant growth than the smallest particle size. Plant biomass usually was the least in the soils amended with the lowest amount of phosphorus (1:5). The treatments in which phosphorus was added in the greatest concentrations did not encourage the largest plant growth. The 1:2 P:As treatment supplied the optimum amount of the nutrient to plants to maximize plant growth. The smallest particle size (<105 µm) usually was associated with the least plant biomass, regardless of the P:As.

Phosphorus deficiencies can significantly reduce plant growth. Rock phosphate is sparingly soluble in alkaline soils, but the ferns of the *Pteris* species have the ability to solubilize rock phosphate in unfavorable conditions. Crystalline apatite is the most stable phosphate mineral in alkaline soils and would be sparingly soluble in the experimental soil [65]. Because of this, the apatite would need to be abundant and finely ground to provide the necessary amount of phosphorus to prevent phosphorus deficiency symptoms. *P. cretica* has the ability to solubilize the apatite [22], and this capability is evident in the biomass data.

A possible explanation for the data in Fig. 3-1 is that the smallest particle size (<105 μm) allowed very rapid dissolution followed by reprecipitation before the plants were able to fully utilize the P released from the apatite. The dissolution kinetics of the largest particle size (250-500 μm) could not provide enough P for maximum growth. The intermediate size fraction (105-250 μm) dissolved quickly enough to meet plant demands but slowly enough to avoid refixing of the P.

3.3.1.2. Plant Arsenic Concentration Affected by Treatment

The plant arsenic concentration follows a similar pattern as biomass. Plants with the intermediate rates of P (1:2) ground to 250-500 μm and 105-250 μm had statistically equivalent As concentrations and were significantly greater than plants in the unamended controls and the finest ground apatite added at the lowest rate (1:5). Plants amended with the highest rate of apatite (1:1) had statistically greater As contents than plants growing in pots with the finest ground apatite added at the lowest rate (1:5).

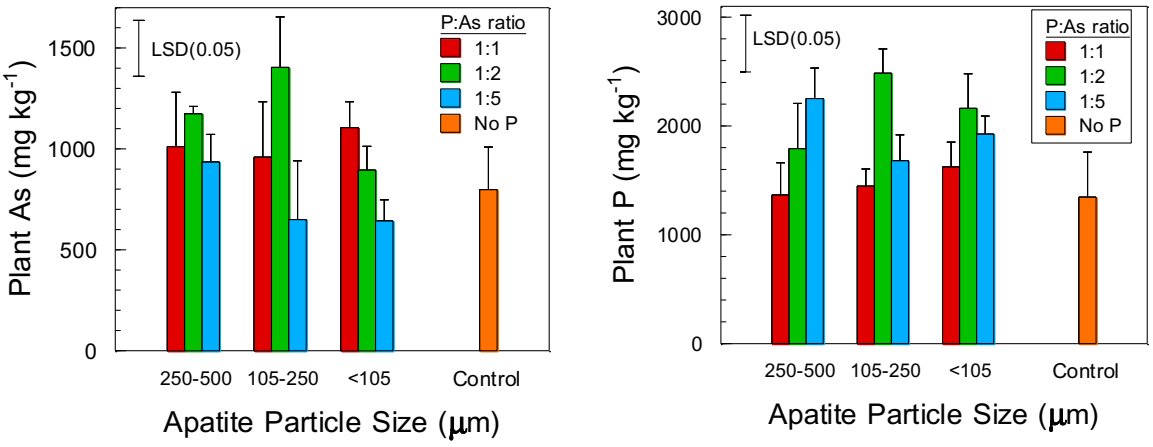

Figure 3-2: Arsenic and phosphorus concentrations in *P. cretica* as affected by P rate and particle size.

These data support the notion that *P. cretica* is able to effectively solubilize at least some portion of the apatite regardless of the particle size. The smallest particle size for all P:As ratios are likely being solubilized most rapidly, but this is not reflected in an increased plant biomass or elevated plant arsenic concentration. The higher rate of dissolution caused by the increased surface area of the small particle size may be resulting in excess phosphorus being fixed by the alkaline soil, resulting in reduced biomass (Fig. 3-1) and lower As concentrations in the plant (Fig. 3-2). The largest particle size has the slowest dissolution rate resulting in the slowest release of phosphorus resulting less-than-optimal plant biomass (Fig. 3-1) and As concentrations significantly less than the highest concentrations (Fig. 3-2). The intermediate particle size (105-250 μM) appears to be the perfect balance between higher kinetics due to the increased surface area, but not to the point of exceeding the plant's ability to assimilate the nutrient and arsenic.

The trends in plant P concentrations (Fig. 3-2) are similar in many ways to the trends in As concentrations, but there are some differences. The 1:2 P:As rate of P application again tends to have higher P concentrations than the 1:1 rates, but the 1:5 rates are associated with higher plant P than the 1:1 and equivalent to the 1:2 rates.

Phosphorus and arsenic share the same receptor in the roots. If one of the elements is excessively higher than the other, then competition for uptake may occur; but, this competition is not expected when solution concentrations of As and P are similar. *P. vittata* plants exposed to elevated As concentrations in nutrient solution were found to have increased biomass, and plants exposed to lower As had less biomass [78]. While it may be tempting to infer that higher concentrations of arsenic (not an essential nutrient) cause the plant to increase its size [83], the data in Figs. 3.1 and 3.2 suggest that greater biomass encourages the plant more arsenic, not the other way around.

A possible explanation for the observed trends in Figs. 3.1 and 3.2 is that the high release of P in the 1:1 treatments can mobilize enough arsenic to cause some arsenic toxicity. *P. cretica* is slightly less resistant to arsenic toxicity than *P. vittata* [11]. As such, the plant might face stunted root growth or the plant may downregulate its phosphorus transporters in an effort to exclude some of the bioaccessible arsenic.

The 1:2 P:As treatment produced plants that simultaneously had the greatest concentration of arsenic and phosphorus. This result is not consistent with the theory that P and As compete for uptake at these levels of P:As and thus leads to one or the other element being preferred. This will be pursued further in the next section.

3.3.1.3. Arsenic Uptake as Affected by Treatment

The potential of *Pteris* species as phytoremediators of As-contaminated soils has been studied extensively. The most effective species are able to remove significant quantities of As from the soil by accumulating high As concentrations in their biomass. The total mass of As removal from the soil by the plants is the result of the combination of high biomass and high concentrations. A direct measure of this is plant As uptake, calculated by multiplying the biomass by the plant concentration.

Plant uptake of arsenic in this study was calculated and plotted in Figure 3.3. As with biomass and plant As concentrations, the plants with the greatest uptake of As were those treated with an intermediate rate of P (1:2) and 105-250 μm particle size (Fig. 3.3). The treatments associated with the lowest As uptake were again the unamended control and the plants receiving the lowest rate of P (1:5) ground to 105-250 and <105 μm particle size. These observations mirror those seen in the other plant data concerning arsenic concentration and plant biomass [78]. The differences between treatments are larger for plant As uptake than As concentrations because the uptake is the product of multiplying concentration by the abovegroind biomass, and the trends in biomass and concentration are similar.

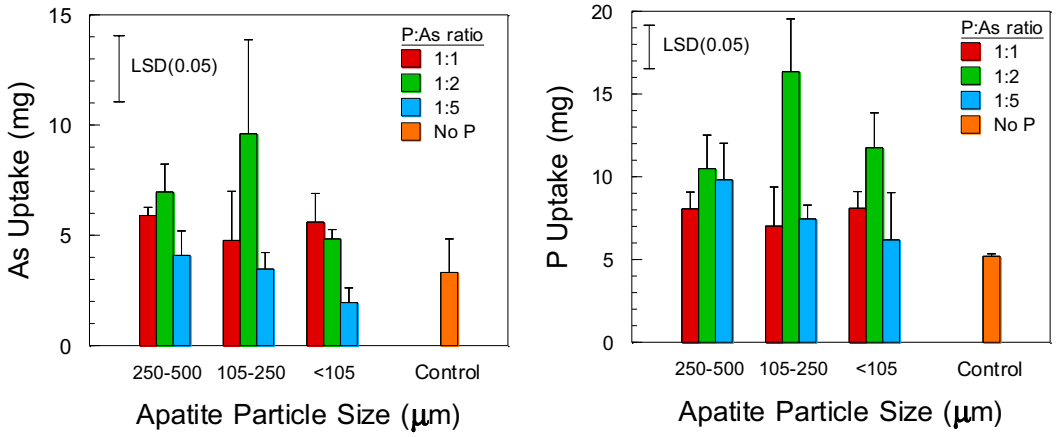

Figure 3-3: Mass of As and P assimilated by *P. cretica* as affected by P rate and particle size.

Plants with the highest masses of arsenic in their tissues will have the greatest impact on the mass arsenic removed from the rhizosphere soils. Those plants with the lowest masses of arsenic in their biomass will have the least impact. The plants with the highest mass of As (1:2 P:As ground to 105-250 μm) removed more than 4.5x the mass removed by the least effective plants (1:5 P:As ground to <105 μm).

Uptake of P by *P. cretica* was determined and follows trends similar to those of As. Phosphorus is an essential plant nutrient and its acquisition is necessary for plant growth and propagation. The highest concentration of phosphorus found in the 1:2 P:As produced plants that had healthy 0.2% concentrations of phosphorus in their tissues. These conditions encourage plant growth and facilitate the uptake of both the phosphorus and arsenic.

The similarity in the trends of the treatment effects on plant biomass and plant As uptake suggests a possible correlation. This was tested in Fig. 3-4. Plant uptake in mg of

both P and As is plotted against plant biomass, and the plant arsenic concentration closely follows that of the plant biomass; both regressions have high R^2 values. In Fig. 3-4 on the right, uptake of As is plotted versus uptake of P, and the regression equation has an R^2=0.42. Figure 3-3 suggests that competition for root uptake between P and As is not an issue in this study, and P is taken up in slightly greater quantities than As.

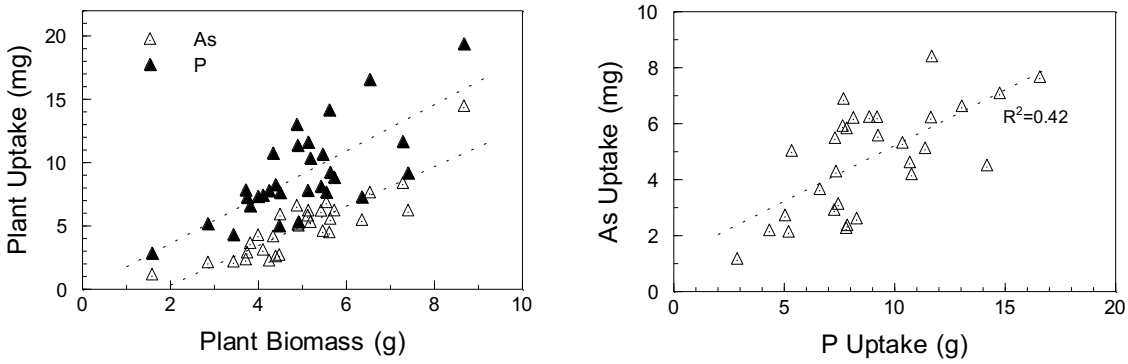

Figure 3-4: Plant uptake of P and As. For the regression equations (dashed lines) R2=0.89 for As and R2=0.57 for P. RIGHT: P uptake versus As uptake.

3.3.2. Soil

3.3.2.1. Arsenic and Lead Content of the Soils

Total lead and arsenic contents of the original, contaminated soil samples were estimated to be 66,440 mg kg^{-1} and 7,520 mg kg^{-1}, respectively, using a field portable XRF. The contaminated soil was then diluted 1:10 (1-part contaminated soil and 9 parts uncontaminated) to bring the projected concentrations of arsenic and lead to more manageable levels of contamination of approximately 6,640 mg kg^{-1} and 752 mg kg^{-1}. To verify the concentrations of As and Pb, the samples were digested using EPA method 3050B and then analyzed by ICP-MS to quantify total concentrations of As and Pb. The average levels of the two contaminants were 523 mg kg^{-1} arsenic and 4610 mg kg^{-1} lead.

The total concentrations were measured for every pot in this experiment and serve as the basis for all calculations involving total Pb and As.

Using total arsenic concentrations in the soils and mass of the rhizosphere soil (mean value 190 g), the mass of arsenic present in the rhizosphere soil can be calculated. Also, the fraction of As removed from the rhizosphere soil can be determined as the ratio of mg As in the plant tissue (plant uptake) and the mass of As in the soil.

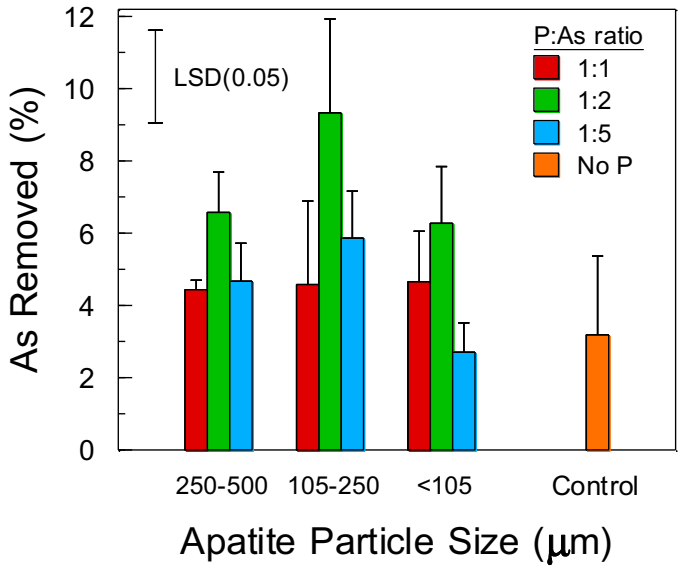

Figure 3-5: Percent As in the rhizosphere soil removed by P. cretica.

The fraction of As removed from the soil by the plants is considerable, and the values are statistically different from each other (Fig. 3-5). The most efficient plants were grown in the treatment with 1:2 P:As ground to 105-250 μm and removed almost 10% of the arsenic in the rhizosphere. The least efficient plants were those grown in the treatment with 1:5 P:As ground to <105 μm but still removed 3% of the total As in the soil. Although this is more than 3x less As than that removed in the highest treatment, a

3.7% percent reduction has been seen in previous trails, in a 12-week timeframe, using the more well-known *P. vittata* [66].

An interesting observation is that treatment with 1:1 P:As (250-500 µM) which had the second highest concentration of arsenic and high plant biomass ranked only sixth among the treatments for total As removed. This is because the total As concentrations in the soils in this treatment were higher than in other treatments and, thus, the percentage of arsenic removed is diminished.

3.3.2.2. Effect of Treatments on IVBA Arsenic

All soils were extracted using the In Vitro Bioaccessibility Assay (IVBA) to assess the effects of treatments on the bioaccessibility of As. The trends for IVBA -As the rhizosphere soils follow a much different pattern than that of the arsenic concentrations in the plant (Fig. 3-6).

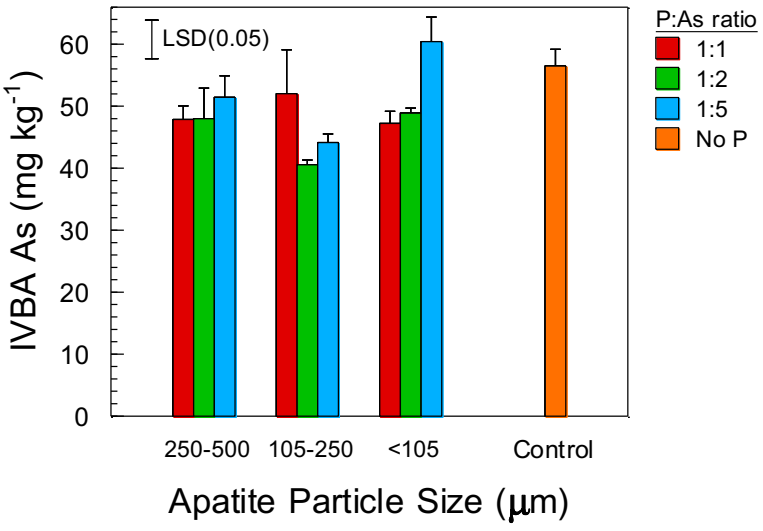

Figure 3-6: IVBA As concentrations in rhizosphere soil.

The treatment with the lowest mean IVBA As concentration was amended with a apatite at a 1:2 P:As ratio ground to 105-250 μm. This treatment produced the largest As concentration in the plants and greatest plant biomass. The treatment with the greatest IVBA As is the 1:5 P:As ground to <105 μm. This treatment had the lowest concentration of arsenic in the plants and was among the lowest plant biomass.

Figure 3-6 seems to infer an inverse correlation between IVBA As concentrations and As plant uptake. Given the significant mass of As removed by the plants from the soil (ranging from 3 to 9% of the total As in the rhizosphere [Fig. 3-5]), reduction in bioaccessible As through plant uptake seems possible. This inverse relationship between IVBA accessible arsenic and removal of As from the soil by the plants was investigated through a regression analysis of plant As uptake on IVBA As (Fig. 3-7). The resulting R value was -0.69 yielding a coefficient of determination of R^2=0.48, indicating that nearly 50% of the variability in IVBA As can be explained by plant uptake of As from the soil by the *P. cretica* plants. This also implies that the *P. cretica* plants growing in this soil are accessing As from the same pool as the IVBA extractant.

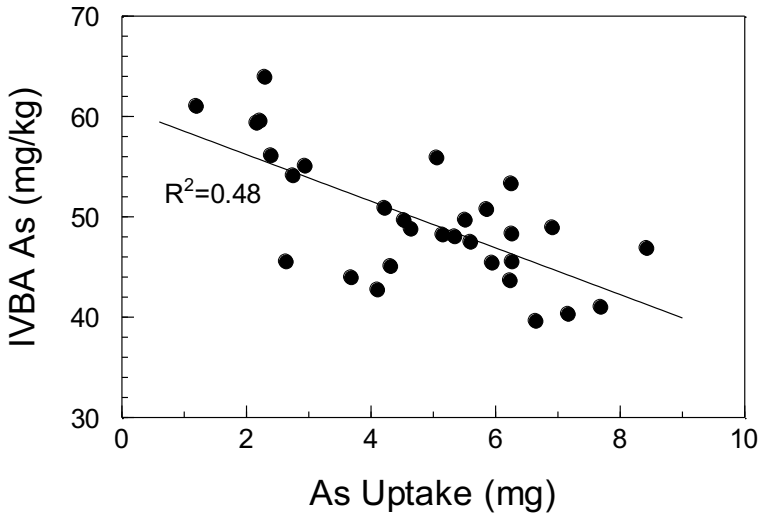

Figure 3-7: Plant uptake of As concentrations vs IVBA As concentrations in the rhizosphere soils.

The combination of plants and treatments did not have the anticipated impact on the bioaccessible As. Phosphate was added specifically to mobilize the arsenic to enhance P uptake. Our hypothesis was that this would have the side effect of increasing the soluble arsenic in the soil and register as increased IVBA As concentrations. This was not the trend, and the opposite was true. In the treatments that encouraged the plants to uptake the most arsenic, and thus the treatments that mobilized the arsenic, the concentrations of bioavailable arsenic are the least. The treatments with optimum phosphorus rates and particle sizes for the plant removal of arsenic depleted the most labile arsenic in the system, causing the bioaccessible portion of the arsenic to be reduced. Although this was not the anticipated result, it is a positive outcome in terms of soil remediation.

A different effect was observed in the soils of the parallel experiment, conducted in the absence of plants. The soils in the parallel experiment were extracted using the

IVBA technique to investigate the concentration of bioavailable arsenic. The trends in Figure 3-8 diverge from the trends that are seen in the rhizosphere soils (Fig. 3-6).

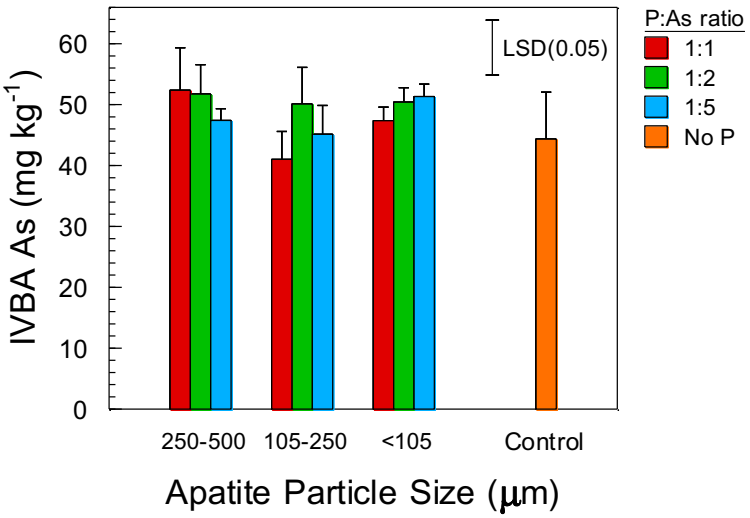

Figure 3-8: IVBA As concentration in the parallel soil.

In Fig. 3-8, little separation exists between IVBA As concentrations as affected by treatment. The analysis of variance found that when determining mean separation with the Student-Newman-Keuls test (a very conservative test), no statistical differences were found. When using the least significant difference (LSD), only the highest and lowest means were different from each other. The lack of statistical significance between treatments signifies that the differences observed in the planted soils were dependent on the influence of the roots. The conclusion from these observations is that the soils alone (in the absence of plants) have little ability to impact the dissolution of apatite and, therefore, do not alter the As soil chemistry.

The chemistry of the soil, being alkaline and clayey, would have limited effect on the chemistry of the added phosphorus due to the stability of apatite in an alkaline soil;

very little phosphate would be released from the particles regardless of the surface area of the particles. Without any apatite being mobilized, no additional phosphate ions would be released to replace arsenic on the sorption sites. This would explain why the bioaccessibility of arsenic does not increase despite the 3 months of incubation in unplanted soils. Even adding a finely ground apatite at 1:1 P:As molar ratio, which if soluble would enhance the bioavailable As, did not have a measurable impact. Without the outside influence of the plants, the soil is unable to solubilize phosphorous, and the amendments have no effect on the bioaccessible concentration of arsenic in the soil.

3.3.2.3. Effect of Treatments on IVBA Lead

Because the soils were more contaminated with both Pb and As (523 mg As/kg and 4610 mg Pb/kg after dilution) and because IVBA has been calibrated for Pb as well as As [63], the soils were analyzed for IVBA Pb. This would quantify any effects that the treatments would have on the bioavailability of lead to humans if the soil is ingested.

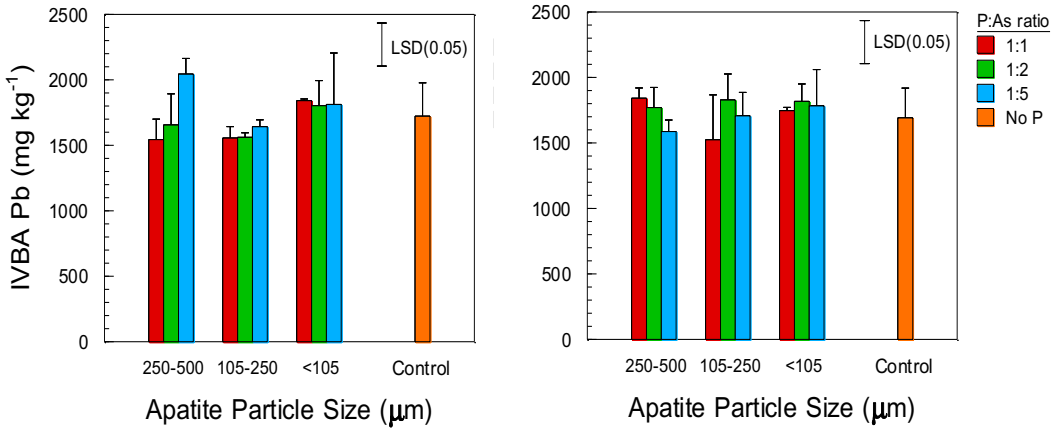

Figure 3-9: Bioavailable lead concentration of the soils in the rhizosphere of the planted experiment as determined by the IVBA extraction. IVBA Pb concentrations in the unplanted soils are on the right.

Unlike the IVBA As, no trends are discernable for IVBA Pb in the rhizosphere soil (Fig. 3-9) and none of the differences are statistically significant. Even though very different levels of phosphorus were added, there were no significant differences in IVBA Pb.

When readily soluble phosphorus is added to a Pb-contaminated soil, the orthophosphate combines with the Pb to form insoluble pyromorphite minerals [47]. The lead pyromorphites are sparingly soluble and their formation will reduce the bioavailability of the metal [53]. However, despite the three different levels of phosphorus additions, there are no differences in the bioavailability of lead among the treatments. The lack of suppression of IVBA Pb despite suppression of IVBA As in some of the treatments is due to two factors: 1) Pyromorphite formation is most favorable at low soil pH and less favorable at the high pH of the experimental soil [49]; and 2) The phosphorus was added in ratios relevant to the arsenic, and inadequate phosphorus concentrations were added to affect the much greater concentrations of Pb.

3.3.2.4. Bicarbonate Extractable Arsenic and Phosphorus

The sodium bicarbonate (pH=8.2) extraction method is used as an index of availability for P in calcareous soils [79]. The bicarbonate ion reacts with Ca from calcium phosphates, precipitates the Ca as $CaCO_3$(calcite), and solubilizes the P. The bicarbonate method also correlated with As availability to plants in one study [80], which is consistent with the similarity in the chemistry of arsenate and orthophosphate. In this study, the hope was that the bicarbonate test could give an indication if a residual benefit existed after growing *P. cretica* in soils amended with mineral that provides no P to most plants. The data gathered by this analysis would show if the As extracted from the soils by the Olsen method would correlate with the arsenic concentrations in the plants.

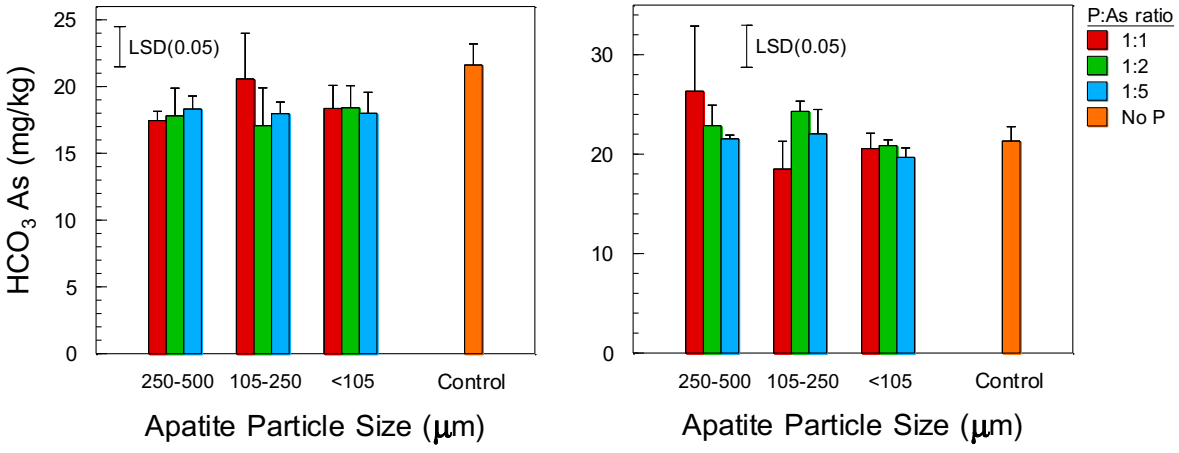

Figure 3-10: Bicarbonate extractable As in the rhizosphere soil (left) and parallel experiment (right).

The bicarbonate As showed no trends as a function of treatment (Fig. 3-10), and according to the Student-Newman-Keuls means separation test, no statistical differences

were found between means of the treatments. While the greatest concentration of arsenic was found in the unamended control, the mean was not statistically different from any other means in the group. The wide range of phosphorus additions and the considerable differences in the size of the phosphorus added had no effect on the sodium bicarbonate available arsenic content on the soil. Likewise, the regression between bicarbonate-As and plant As concentrations was not significant (R^2=0.02).

The soils in the parallel (unplanted) experiment were extracted using the sodium bicarbonate method to determine plant available arsenic. The only significant difference was between the two largest mesh sizes for the 1:1 P:As treatment. Therefore, although *P. cretica* was able to solubilize both As and P from the rhizosphere, the changes in chemistry imposed by the roots were temporary and not reflected in the bicarbonate index.

The sodium bicarbonate method also was used to evaluate the impact of apatite treatments on the bioavailability of P in rhizosphere and unplanted soils (Fig. 3-11).

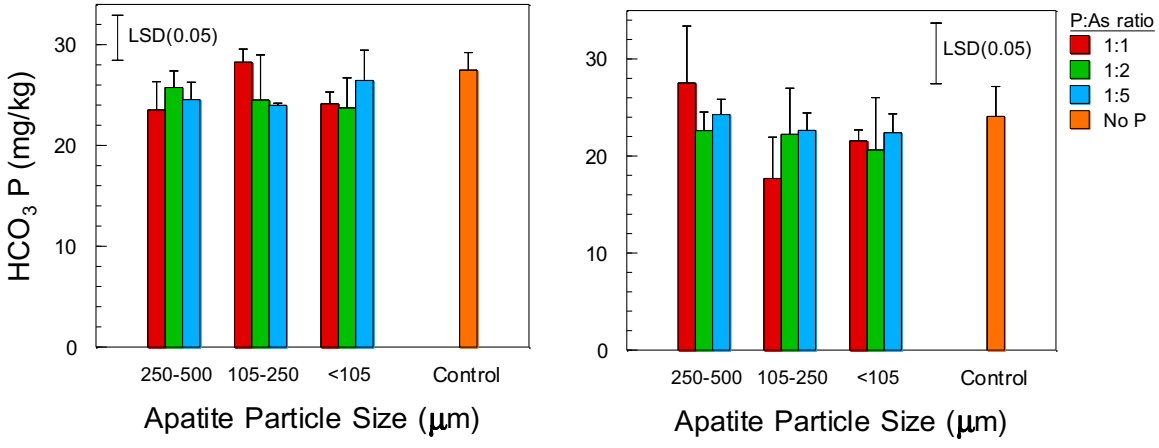

Figure 3-11: Effects of treatments on bicarbonate P in rhizosphere soil (left) and unplanted soil in the parallel experiment (right).

As with the arsenic sodium bicarbonate data, there is little separation between the means of the phosphorus concentrations regardless of treatment. For the rhizosphere soil data (Fig. 3-11 left), despite the influence of the plant roots on the soils, none of the treatment effects were significant. The phosphorus in the treatments was added as sparingly soluble apatite which only releases trace amounts of phosphate in an alkaline environment.

The apatite treatments on had little impact on HCO_3-P; HCO_3-P was not correlated with plant P; HCO_3-As was not correlated with plant As; and plant biomass was not related to HCO_3-P. However, the relationship between the assimilated mass of P or As and plant biomass is clear (Fig. 3-4), verifying that the chemistries of phosphate and arsenate are tied. This relationship is further implied when examining the correlation of rhizosphere HCO_3-P with HCO_3-As (Fig. 3-12) in which the relationship has a R of 0.64 and coefficient of correlation of 0.41. This relationship is not observed in the bulk soil (R^2=0.10) or the soil from the unplanted experiment (R^2=0.15), indicating that the plant roots altered the chemistry of P and As in a similar way.

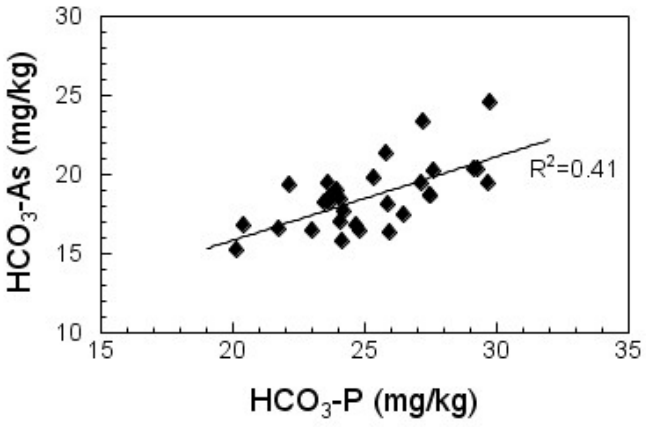

Figure 3-12: Bicarbonate P vs Bicarbonate As in rhizosphere soils

Pteris cretica has the ability to break down the apatite particles and free phosphate ions through acidification or exudation of organic complexing agents [19], but very little of the phosphate will remain soluble. Alkaline soils frequently are deficient in plant available phosphorus due to many ways that the phosphorus can be "fixed". Once phosphorus is released, it must be assimilated by an organism or it will be quickly sequestered. Root exudates are a primary mechanism of mobilization of phosphorus from apatite and thus phosphate ions are in close proximity to the plant roots to be taken up into the plant [30]. In the experimental soil, mobilized phosphate ions will react rapidly with lead to form insoluble pyromorphite species with very low solubility and low bioavailability. Because of the properties of the soil and the low availability of apatite in these soils, bicarbonate extraction is not a good model for phosphorus bioavailability for *P. cretica.*

3.4. Summary and Conclusions

The addition of apatite in an arsenic and lead co-contaminated soil had a significant impact on the bioavailability and concentration of arsenic in the rhizosphere of *Pteris cretica*. Some of the treatments had a significant impact on plant's concentration and mass of arsenic adsorbed. The most optimum treatments enabled the plant to remove as much as 10% of rhizosphere's arsenic content in three months of growth. The same treatments also decreased the concentrations of bioaccessible arsenic, as opposed to the control and the least optimum treatments. In addition, the use of the apatite amendment encouraged the plants to grow to larger sizes and facilitated an increase in the concentration of the arsenic. The intermediate rate of phosphorus amendment with the two smallest particle sizes were the most effective treatments. Over successive harvests and a longer time frame, the plants in combination with the treatments would make a large impact on the arsenic contamination of the soil. If the plants were allowed full growth cycles, their roots would explore a greater fraction of the soil volume and would be effective in phytoremediating the contaminated soil.

Amending the soil with apatite had no impact on the bioaccessibility of lead. Apatite has been shown to facilitate the immobilization of lead via dissolution of the mineral and then the precipitation of immobile pyromorphite, and this mechanism effectively removes the lead from the bioaccessible realm as shown in IVBA tests. None of the treatments in this study had any effect on bioaccessible lead fraction because the amount of phosphorus used in this trial was in very small quantities compared to the levels of lead present and thus could only have a marginal effect on the bioaccessibility

of the metal. In this scenario, due to the greatly higher levels of lead compared to arsenic, apatite applied at these rates is not a viable way to immobilize the lead. The most optimum rate of phosphorus to lead addition is 3:5 which using the apatite necessitate massive amounts of the mineral added to the soil [67]. High applications of phosphorus would also start to compete with the arsenic for plant uptake and could have the impact of increasing the human available arsenic concentrations while lowering the amount of arsenic removed by the plant.

A shortcoming of this experiment was the lack of uniformity of the stored soil samples. After the plants grew and the soil was analyzed, there was a clear distinction in the concentration of the arsenic contamination in the soils from the two storage bins used in this experiment. The soil in the first bin was exhausted midway in the preparation of the 1:5 dilution, and that created insurmountable impediments in analyzing the data from that dilution series. Therefore, the data were not used in this thesis.

4. CONCLUSIONS

This thesis presents results from a series of experiments conducted on a highly contaminated soil that was sampled from the Jacobs Smelter Superfund site. This area is the former site of several smelters that began operations in the 1860s and ran for nearly 100 years. The soils from this site are contaminated with arsenic and lead with concentrations that pose a significant risk to human health and environment. In this thesis we studied the mobilization of arsenic for plant uptake and the immobilization of lead caused by different rates and particle sizes of apatite addition. The treatments were composed of three different rates of phosphorus addition, and three different particle sizes of the apatite. The source of the phosphorus was the mineral apatite, meant to release the nutrient into the system slowly and without overwhelming the system. Apatite, being only sparingly soluble in alkaline systems, would not release all of its phosphorus immediately unlike soluble sources thus preventing plant death from salt toxicity and eutrophication from runoff.

In post-harvest analysis of the effectiveness of the phosphorus amendments, an inverse relationship was discovered between the amount of arsenic removed from the soil by the plant and the concentration of bioaccessible arsenic in the soil as determined by a IVBA test. The bioaccessible arsenic concentrations decreased by a significant amount of nearly 10% for the most effective treatments found in the 1:2 P:As ratio and the two largest particle sizes. Also observed was an increase in plant biomass was correlated with an increase in the concentrations of arsenic and phosphorus within the plant. Phosphorus has been shown to promote plant growth and thus amplify the uptake

of both arsenic and phosphorus. Over successive harvests, the concentration of arsenic in the soil can be brought to below toxic levels and successfully remediate a contaminated site. However, lead bioavailable levels were not impacted by any additions of phosphorus because the P was not added in high enough concentrations to impact the much higher levels of lead in the system as compared to arsenic.

The need for alternative methods for treatment of As and Pb polluted soil calls for continued research to find the most economical and efficient technologies. Arsenic mobilization for phytoremediation and lead stabilization with the use of phosphate may work for many different sources of contamination, not only mining and smelting contaminated soil including soil contaminated with excess use of pesticides, wood preservation process as well as soil contaminated surrounded in industrial manufacturing plants.

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TEXAS A&M AGRILIFE EXTENSION

Soil Analysis Report

Soil, Water and Forage Testing Laboratory
Department of Soil and Crop Sciences
2478 TAMU
College Station, TX 77843-2478
979-845-4816 (phone)
979-845-5958 (FAX)
Visit our website: http://soiltesting.tamu.edu

Report generated for:
Andrew Lee
2474 TAMU
COLLEGE STATION, TX 77845

Sample received on: 5/1/2020
Printed on: 5/8/2020
Area Represented: not provided

Crockett County
Laboratory Number: 556176
Customer Sample ID: Ozona
Crop Grown: NO CROP GIVEN

Analysis	Results	CL*	Units	ExLow VLow Low Mod High VHigh Excess.
pH	7.8	(5.8)	-	Mod. Alkaline
Conductivity	258	(-)	umho/cm	None — Fertilizer Recommended
Nitrate-N	4	(-)	ppm**	
Phosphorus	17	(0)	ppm	
Potassium	550	(0)	ppm	
Calcium	16,768	(180)	ppm	
Magnesium	189	(50)	ppm	
Sulfur	15	(13)	ppm	
Sodium	323	(-)	ppm	
Iron				
Zinc				
Manganese				
Copper				
Boron				
Limestone Requirement				

Textural Analysis Test (hydrometer)		
Sand	25	%
Silt	38	%
Clay	37	%
Textural Class:	Clay Loam	

Organic Matter	3.02	%

*CL=Critical level is the point which no additional nutrient (excluding nitrate-N, sodium and conductivity) is recommended. **ppm=mg/kg

New online fertilizer calculators have been placed on the laboratory's website to determine appropriate fertilizers to purchase and determine their application rates. http://soiltesting.tamu.edu/webpages/calculator.html

Methods: pH and conductivity/ 2:1; nitrate-N/Cd-red.; P, K, Ca, Mg, Na, and S/Mehlich 3 by ICP; Fe, Zn, Mn, and Cu/DTPA by ICP; and B/hot water by ICP.

ProAnalysisVer. 2.19j

Figure A-1: Soil testing report for Ozona series soil.

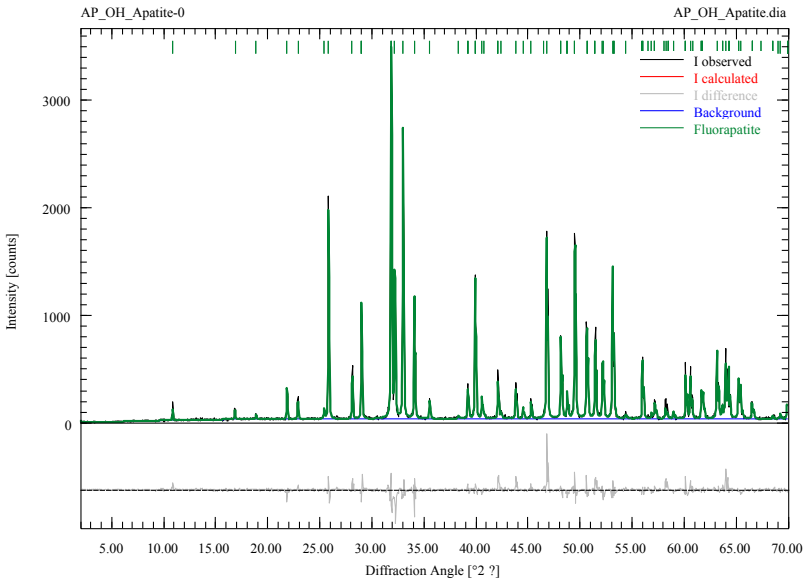

Figure A-2: XRD analysis on apatite used.

Dilution	P Add	Mesh	TRT	totalAsR	totalPbR	totalAsB	totalPbB	totalAsX	totalPbX	pbetAsR	pbetPbR	pbetAsB	pbetPbB	pbetAsX	pbetPbX	HCO3AsR	HCO3PbR	HCO3PR	HCO3AsB	HCO3PbB	HCO3PB	HCO3AsX	HCO3PbX	HCO3PX	Plant As
10	0	0	1	845	4960	834	4807	633	5751	59.4	1992.8	48.8	1178.9	48.7	1774.0	212	50.7	25.8	27.7	54.7	293	20.4	55.4	213	755
10	0	0	1	552	3614	685	4631	415	3988	54.2	1697.2	52.4	1291.3	35.4	1433.0	202	47.0	29.3	29.9	67.2	313	23.0	62.5	23.6	612
10	0	0	2	474	3503	619	4398	623	5543	56.0	1483.8	54.7	1398.0	48.9	1863.3	233	40.7	27.2	31.3	70.0	283	20.6	57.9	27.4	1027
10	1	35	2	698	4691	591	3660	716	6337	49.8	1568.5	42.3	978.4	45.2	1803.0	167	47.5	20.4	20.1	45.4	304	32.4	67.5	31.4	866
10	1	35	2	693	4449	655	4279	689	6442	45.5	1374.2	40.5	858.5	59.2	1930.7	180	48.4	25.9	25.3	58.6	354	19.3	49.2	30.4	1321
10	1	35	2	707	4370	562	4428	790	6757	48.4	1685.4	40.1	983.0	52.6	1786.0	176	47.2	24.2	19.6	47.9	336	27.3	55.8	20.8	844
10	1	60	3	463	3497	518	4003	370	3503	50.8	1567.8	46.8	1056.9	36.7	1551.4	186	47.0	27.5	24.6	58.1	410	15.6	39.4	13.3	1141
10	1	60	3	558	4083	528	3933	454	4655	59.6	1634.5	56.7	1215.8	30.6	1165.8	245	56.5	29.8	31.8	59.6	490	18.7	42.8	18.0	643
10	1	60	3	657	4052	698	4125	563	5049	45.6	1459.8	52.3	1211.0	45.8	1851.9	186	44.2	27.5	21.0	43.4	395	21.2	48.4	21.7	1093
10	1	140	4	673	4753	547	3959	716	6434	49.0	1855.3	48.9	1267.6	48.9	1725.2	190	48.5	23.9	26.7	62.7	417	21.8	55.1	20.3	1245
10	1	140	4	532	3707	609	4218	681	5949	47.6	1828.8	45.5	1110.1	48.4	1778.8	164	40.9	23.0	28.6	62.6	534	21.0	49.7	21.9	992
10	1	140	4	746	4607	663	4543	675	6636	45.1	1836.1	55.1	964.9	44.9	1738.8	164	40.9	23.0	28.6	62.5	534	21.0	49.7	21.9	1078
10	0.5	35	5	621	4158	685	4543	565	5596	53.4	1922.2	46.5	1631.5	46.8	1605.5	202	47.4	27.6	29.6	62.6	418	21.8	51.7	20.6	1216
10	0.5	35	5	608	4497	563	4339	634	5647	46.9	1578.0	46.1	1927.4	51.8	1911.7	168	48.4	24.7	27.2	60.5	290	21.5	49.6	24.4	1156
10	0.5	35	5	458	3786	508	4129	642	5627	43.7	1462.3	44.3	1546.1	56.5	1790.4	164	42.4	24.8	31.3	71.9	571	25.2	50.7	22.9	1148
10	0.5	60	6	647	4805	520	4080	412	4318	40.9	1520.6	50.3	1127.5	51.6	1834.6	203	53.3	29.1	27.4	62.1	345	23.1	52.3	27.2	1362
10	0.5	60	6	529	4047	456	3642	451	4426	39.7	1571.3	43.5	1331.3	44.9	1624.0	151	44.9	20.2	27.2	62.8	345	23.1	52.3	27.2	1362
10	0.5	60	6	421	3823	445	3709	700	6127	41.1	1587.7	44.3	1038.8	55.2	2022.0	157	43.6	24.2	27.1	64.0	334	24.8	52.9	21.7	1175
10	0.5	140	7	474	3650	326	4856	783	5162	49.7	2003.9	47.9	1373.2	53.0	1968.2	165	47.0	21.8	29.2	67.3	347	20.5	44.7	26.7	805
10	0.5	140	7	349	3120	534	6165	628	4372	48.1	1630.9	45.6	1118.8	49.9	1762.2	193	45.2	22.1	33.6	68.3	380	20.5	48.5	18.6	1029
10	0.5	140	7	423	3853	297	4762	708	5981	48.8	1781.5	47.0	1489.5	48.3	1720.9	194	52.1	27.2	27.0	58.8	279	21.5	55.8	16.4	848
10	0.2	35	8	486	3910	362	5145	694	6087	48.3	2030.5	42.4	1110.9	49.4	1619.9	174	48.1	26.5	27.6	66.7	296	21.9	50.2	26.0	1050
10	0.2	35	8	440	3912	406	5424	745	6724	55.1	2167.6	43.6	951.9	47.3	1653.6	193	50.6	23.6	28.6	70.7	319	21.2	54.1	23.9	783
10	0.2	35	8	449	3591	467	6056	719	6353	50.9	1929.1	41.9	1285.0	45.5	1486.3	182	46.6	23.5	27.6	68.1	295	21.4	52.9	22.9	971
10	0.2	60	9	315	3271	561	6260	504	4684	45.6	1601.9	43.5	1370.6	45.1	1813.4	185	44.0	23.7	25.1	58.4	298	19.5	61.4	20.6	598
10	0.2	60	9	282	3905	339	5400	580	5295	44.0	1622.8	45.8	1331.1	40.4	1500.0	184	49.6	24.1	17.6	37.8	324	24.4	57.6	23.8	963
10	0.2	60	9	339	5328	533	5332	653	6058	42.8	1700.4	39.5	1133.5	49.9	1805.6	170	44.3	24.1	24.1	54.3	282	22.1	49.7	23.5	387
10	0.2	140	10	376	5495	344	4249	496	5011	64.0	1730.7	37.1	3251.4	53.7	2074.7	194	29.7	26.1	59.7	32.4	18.6	42.0	21.4	538
10	0.2	140	10	399	5159	381	4466	794	6920	56.2	2239.0	39.5	3495.1	50.4	1520.4	163	45.4	26.0	24.5	54.8	303	20.5	58.2	24.6	642
10	0.2	140	10	354	5432	442	4989	674	6051	61.1	1462.4	39.9	3031.4	49.8	1750.9	182	45.7	23.7	25.9	58.0	296	19.9	51.3	21.1	748
10	0	0	1	583	10114	621	8343	1520	13529	44.0	1936.0	65.6	3222.5	92.8	4386.7	306	102.0	30.3	39.1	142.0	366	29.9	114.0	24.5	581
10	0	0	1	646	11314	593	8301	1340	11761	47.1	2150.8	73.9	2745.5	85.0	4113.7	256	106.3	30.0	39.1	111.5	289	32.4	114.4	26.2	885
10	0.5	35	1	623	10572	561	8240	1309	11337	44.2	2166.2	59.3	2827.4	83.2	3974.6	259	100.1	26.5	40.3	118.7	395	31.6	117.8	27.3	469
10	0.5	35	2	571	9834	603	8502	1532	13610	54.7	2260.1	66.9	2642.4	89.3	3890.2	257	89.0	31.6	43.7	128.8	390	31.0	118.5	25.9	770
10	1	35	2	460	8150	646	10627	1181	11327	37.8	1645.9	71.5	2656.3	78.8	3836.7	287	110.8	34.8	41.0	123.3	433	25.9	88.2	25.7	899
10	1	35	2	540	11218	551	8922	1241	11101	45.8	2046.2	70.3	2828.2	81.4	3546.3	263	92.0	35.9	45.4	125.0	413	23.9	100.2	25.3	1075
10	1	60	3	503	9166	644	9382	1092	9619	40.4	1679.4	67.5	2805.7	80.2	3793.1	446	132.7	32.4	44.1	125.2	429	27.9	104.4	27.3	803
10	1	60	3	885	11106	596	9584	1481	12600	43.0	1981.4	73.9	3035.1	77.4	3503.2	404	116.9	30.7	48.3	132.4	413	27.2	94.5	19.5	1062
10	1	60	3	573	9540	594	9003	1382	12188	56.5	2497.4	77.3	2645.6	73.6	3444.1	440	122.9	29.6	44.1	122.7	387	33.0	96.8	26.8	901
10	1	140	4	403	7815	511	9361	1353	11769	50.8	2167.4	66.1	3495.0	85.2	3654.5	413	147.0	28.3	44.1	98.9	253	33.3	90.6	21.2	551
10	1	140	4	396	6587	560	8519	1365	12348	45.9	1923.0	70.3	3442.4	77.2	3380.6	389	136.5	30.6	38.8	95.3	278	35.0	104.5	23.0	842
10	0.5	35	5	379	6840	527	8931	1479	12867	53.7	2553.4	90.4	3040.3	79.2	3931.1	378	120.7	21.5	45.2	95.9	238	29.0	84.7	32.3	781
10	1	140	4	439	7449	564	10060	1375	12867	50.5	2283.5	77.9	2643.0	73.2	3409.0	431	117.8	24.6	38.1	90.0	370	29.2	87.6	22.9	433
10	0.5	35	5	444	7786	551	8685	1454	12137	62.0	2614.1	71.3	3386.3	92.4	4161.6	542	180.0	32.6	34.7	103.2	372	28.1	92.9	28.0	1150
10	0.5	35	5	441	7524	648	9849	2038	13438	57.1	2415.8	78.5	3212.8	87.9	3854.1	405	113.2	30.1	40.5	112.6	391	24.9	83.5	25.1	342
10	0.5	60	6	516	8190	675	9668	1951	14336	67.3	2951.6	74.0	3049.3	87.9	3854.1	405	113.2	30.1	40.5	112.6	391	24.9	83.5	25.5	816
10	0.5	60	6	305	5472	822	9828	1539	10947	52.2	2142.3	90.0	3355.9	75.7	3439.0	361	129.1	22.1	43.9	107.5	407	26.9	73.9	28.4	481
10	0.5	60	6	452	6072	904	8973	1816	24173	60.8	2818.9	88.7	3007.3	78.2	3858.1	490	176.8	28.0	37.9	95.4	405	22.2	60.8	25.5	1091
10	0.5	140	7	1271	10385	1924	15843	2226	15835	45.8	2349.5	68.0	2970.9	98.6	4234.5	295	108.2	22.3	29.2	74.4	286	23.2	84.9	21.9	1134
10	0.5	140	7	953	7840	2108	12441	1730	12441	48.3	2457.3	82.3	3837.8	85.2	3257.8	305	108.6	22.8	28.5	78.8	220	16.9	51.5	28.0	1277
10	0.5	140	7	1047	8780	1907	11396	1642	11499	58.1	2782.8	69.9	4098.3	77.4	3257.8	286	101.0	25.5	27.6	86.0	281	22.8	76.3	27.4	711
10	0.2	35	8	1133	9106	1484	14937	1828	12223	52.8	2482.9	73.8	3905.9	81.6	3681.3	486	135.6	39.7	34.9	87.2	340	23.3	75.2	24.2	790
10	0.2	35	8	1129	9871	1345	13534	2128	14394	68.8	3045.4	71.8	4080.4	99.7	4276.1	318	103.6	32.9	41.5	96.8	371	22.4	73.6	37.9	1010
10	0.2	35	8	821	6900	1736	14864	1894	13015	49.2	2464.8	76.0	3743.2	82.9	3586.6	378	104.5	30.9	28.5	78.1	299	24.2	75.7	28.7	1086
10	0.2	60	9	955	8342	1314	12610	2048	22749	64.9	3504.9	64.7	5314.5	88.3	3923.6	250	130.9	17.4	23.3	95.1	256	23.7	74.4	26.2	1723
10	0.2	60	9	1321	10303	316	4101	1518	20843	37.6	2055.3	43.6	1680.2	69.3	3045.8	264	128.3	48.3	18.5	38.8	356	21.4	76.4	26.1	1220
10	0.2	60	9	1247	10849	1295	11895	2064	30779	49.1	2587.7	58.4	3617.4	84.1	3473.6	255	123.7	17.6	25.4	104.5	277	24.3	76.1	27.0	743
10	0.2	140	10	424	6331	1541	15710	2071	27051	37.2	1426.1	71.3	4535.1	78.0	3480.0	224	179.2	36.4	18.3	134.6	248	22.0	85.2	30.3	222
10	0.2	140	10	916	8334	1512	14418	2181	24213	46.3	2605.8	70.6	3438.7	72.5	3133.6	210	167.5	16.8	18.4	123.4	212	23.9	82.5	29.3	719
10	0.2	140	10	1128	10007	1448	13404	1632	18535	49.9	2693.2	77.5	3271.4	88.8	4093.3	328	110.1	24.4	18.2	130.9	262	21.7	65.3	25.4	373

Figure A-3: Complete data set of analysis on 1:10 and 1:5 dilutions.